COMPARATIVE EVALUATION OF ANTIMICROBIAL, ANTHELMINTIC AND IMMUNOMODULATORY ACTIVITIES OF *MARTYNIA ANNUA* L. AND *PENTANEMA INDICUM*

A Dissertation submitted to

THE TAMIL NADU Dr. M. G. R. MEDICAL UNIVERSITY

Chennai-600032

In partial fulfillment of the requirements for the award of degree of

MASTER OF PHARMACY

IN

PHARMACOLOGY

Submitted by

REG. No.: 261525551

Under the Guidance of

Mr. P. SUDHAKAR, M. Pharm.,
Assistant Professor, Department of Pharmacology

DEPARTMENT OF PHARMACOLOGY,
SWAMY VIVEKANANDHA COLLEGE OF PHARMACY, ELAYAMPALAYAM,
TIRUCHENGODE-637 205,TAMIL NADU

APRIL-2017

SWAMY VIVEKANANDHA COLLEGE OF PHARMACY
Elayampalayam, Tiruchengode- 637205
Namakkal (DT.), Tamilnadu.
Phone: 04288-234417 (8 lines)
Fax: 04288-234417

Dr. G. MURUGANANTHAN, M. Pharm., Ph.D.,

PRINCIPAL

CERTIFICATE

This is to certify that the Dissertation entitled on **"COMPARATIVE EVALUATION OF ANTIMICROBIAL, ANTHELMINTIC AND IMMUNOMODULATORY ACTIVITIES OF *MARTYNIA ANNUA L.* AND *PENTANEMA INDICUM*"** submitted to **The Tamil Nadu Dr. M.G.R Medical University**, Chennai, is a bonafide project work carried out by **GAYATHIRI N.M (Reg. No.: 261525551)** in the Department of Pharmacology, Swamy Vivekananda College of Pharmacy, Tiruchengode, for the partial fulfillment for the degree of Master of Pharmacy under the guidance and direct supervision of **Mr. P.SUDHAKAR, M.Pharm.,** in the Department of Pharmacology during the academic year of 2016-2017.

Date:

Place: **Dr. G. MURUGANANTHAN, M. Pharm., Ph.D.,**

SWAMY VIVEKANANDHA COLLEGE OF PHARMACY

Elayampalayam, Tiruchengode- 637205

Namakkal (DT.), Tamilnadu.

Phone: 04288-234417 (8 lines)

Fax: 04288-234417

Dr. V.VINOTH PRABHU, M. Pharm., Ph.D.,

Head, Department of Pharmacology

CERTIFICATE

This is to certify that the Dissertation entitled on **"COMPARATIVE EVALUATION OF ANTIMICROBIAL, ANTHELMINTIC AND IMMUNOMODULATORY ACTIVITIES OF *MARTYNIA ANNUA L.* AND *PENTANEMA INDICUM*"** submitted to **The Tamil Nadu Dr. M.G.R Medical University**, Chennai, is a bonafide project work carried out by **GAYATHIRI N.M (Reg. No.: 261525551)** in the department of pharmacology, Swamy Vivekananda College of Pharmacy, Tiruchengode, for the partial fulfillment for the degree of Master of pharmacy under the guidance and direct supervision of **Mr. P.SUDHAKAR, M. Pharm.,** in the Department of Pharmacology during the academic year of 2016-2017.

Date:

Place: **Dr. V. VINOTH PRABHU, M.Pharm., Ph.D**

SWAMY VIVEKANANDHA COLLEGE OF PHARMACY

Elayampalayam, Tiruchengode- 637205

Namakkal (DT.), Tamilnadu.

Phone: 04288-234417 (8 lines)

Fax: 04288-234417

Mr. P.SUDHAKAR, M. Pharm.,

Assistant Professor, Department of Pharmacology

CERTIFICATE

This is to certify that the Dissertation entitled on **"COMPARATIVE EVALUATION OF ANTIMICROBIAL, ANTHELMINTIC AND IMMUNOMODULATORY ACTIVITIES OF *MARTYNIA ANNUA L.* AND *PENTANEMA INDICUM*"** submitted to **The Tamil Nadu Dr. M.G.R Medical University**, Chennai, is a bonafide project work carried out by **GAYATHIRI N.M (Reg. No.: 261525551)** in the Department of Pharmacology, Swamy Vivekananda College of Pharmacy, Tiruchengode, for the partial fulfillment for the degree of master of pharmacy under the guidance and direct supervision during the academic year of 2016-2017.

This work is original and has not been submitted earlier for the award of any other Degree or Diploma of this or any other university.

Date:

Place: **Mr. P.SUDHAKAR, M. Pharm.,**

EVALUATION CERTIFICATE

This is to certify that the Dissertation entitled on **"COMPARATIVE EVALUATION OF ANTIMICROBIAL, ANTHELMINTIC AND IMMUNOMODULATORY ACTIVITIES OF *MARTYNIA ANNUA L.* AND *PENTANEMA INDICUM*"** submitted to **The Tamil Nadu Dr. M.G.R Medical University**, Chennai, in partial fulfillment for the degree of Master of Pharmacy. This was carried out by **GAYATHIRI N.M. (Reg. No.: 261525551)** under the guidance and direct supervision of **Mr. P. SUDHAKAR, M. Pharm.,** in the Department of Pharmacology, Swamy Vivekananda College of Pharmacy, Tiruchengode for the during the academic year of 2016-2017.

Internal Examiner **External Examiner**

Examination centre:

ACKNOWLEDGEMENT

ACKNOWLEDGEMENT

The joy, satisfaction and euphoria that come along with successful completion of any work would be incomplete unless I mention the names of the people who made it possible, whose constant guidance and encouragement served as a beam of light that crowned out effects. I fetch this sensible opportunity to express my heartfelt thanks to all the people who have shaped this thesis.

First and foremost, I bow down before Lord Almighty for his splendid blessings and care in completing my project work and throughout my life till this very second.

I feel it a great honor to express my deep sense of gratitude and indebtedness to my guide. **Mr. P. SUDHAKAR, M. Pharm.,** Department of Pharmacology, thanking for his willingness to offer continuous guidance, support and encouragement, which were the driving forces for me to complete this thesis. His vast knowledge, attitude of research and skill of presentation had been an invaluable resource to me.

It is difficult to overstate my gratitude to **Dr. G. MURUGANANTHAN, M.Pharm., Ph.D.,** Principal of this institution. His enthusiasm and integral view on research and his mission for providing ‘only high-quality work and not less’, has made a deep impression on me. I owe him lots of gratitude for having me shown this way of research.

I feel it a great honor to express my deep sense of gratitude and indebtedness to my Head of Department of Pharmacology of this institution **Dr. V. VINOTH PRABHU, M.Pharm., Ph.D.,** thanking for his support encouragement and his constructive ideas at each and every stage of the project which were the driving forces for me to complete this thesis. His vast knowledge, attitude of research and skill of presentation had been an invaluable resource to me. He is an admirable professor and will always be a role model for me.

Generally, foundations are not visible anywhere. But it is the foundation that holds everything at place. I render my sincere thanks to our Honourable **Chairman &**

Secretary, Vidya Rathna, Rashtriya Rathna, Hind Rathna Prof. Dr. M. KARUNANITHI, B.Pharm.,M.S., Ph.D., D.Litt., for providing all facilities for our study and rendering his noble hand in the upliftment of women education in all disciplines.

I owe my sincere thanks to **Mr. R. Anand, M. Pharm., (Ph.D)., Ms. S. Priyadharshini, M.Pharm., Ms. K. B. Suchithra. B. Pharm.,** Department of Pharmacology for their immense support during the course of my project.

I also thank pharmacology lab assistant's **Mrs. L. Sathiya, Mr. V. Karunakaran, Mr. M. Parameshwaran, Ms. S. Gowri** for their help during my project work.

I owe my heartfelt gratitude to my respected Parents **Mr**.**S. Muthusamy, Mrs. M. Kalaichelvi,** who cared for my well-being and had spent their times in shaping my character, conduct and my life. Without their moral support, I am nothing and I dedicate all my achievements at their feet.

Friends are treasures to me and it is very difficult to overstate my thanks to all my friends. It has been my happiest time to study, discuss, laugh and play with them all. I express my whole hearted thanks to my friends.

I would like to thanks **The Tamil Nadu Dr. M. G. R. Medical University** for providing a nice environment for learning.

I fell delighted to express my whole hearted gratitude to all those who gave their helping hands in completing my course and my project successfully.

N.M.GAYATHIRI

(Reg.No: 261525551)

CONTENT

Contents

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

WHO	_	World Health Organization
E.coli	_	*Escherichia coli*
S.aureus	_	*Staphylococcus aureus*
P.aerugenosa	_	*Pseudomonas aerugenosa*
B.subtilis	_	*Bacillus subtilis*
M.luteus	_	*Micrococcus luteus*
MIC	_	Minimum Inhibitory Concentration
MBC	_	Minimum Bactericidal Concentration
C.albicans	_	*Candida albicans*
C.kruzei	_	*Candida krusei*
ZI	_	Zone of Inhibition
A.niger	_	*Aspergillus niger*
MA	_	*Martynia annua* L.
PI	_	*Pentanema indicum*
HAEMA	_	Hydroalcoholic Extract of *Martynia annua* L.
HAEPI	_	Hydroalcoholic Extract of *Pentanema indicum*
PI	_	Phagocytic Index
SEM	_	Standard Error Mean
mg	_	milligram
ml	_	millilitre
µl	_	microlitre
min	_	minutes
h	_	hour

g/L	_	gram per litre
mg/ml	_	milligram per millilitre
%	_	percentage
PBS	_	Phosphate Buffer Solution
GAA	_	Glacial Acetic Acid
H_2SO_4	_	Sulphuric acid
$FeCl_3$	_	Ferric chloride
CT	_	Condensed tannins

ABSTRACT

ABSTRACT

Title	:	**Comparative Evaluation of Antimicrobial, Anthelmintic and Immunomodulatory activities of *Martynia annua* L. and *Pentanema indicum***
Name	:	**Gayathiri.N.M**
Degree to which submitted	:	**Master of Pharmacy in Pharmacology**
Guide	:	**Mr. P. Sudhakar, M. Pharm.,**
Department	:	Department of pharmacology
College	:	Swamy Vivekanandha College of Pharmacy, Tiruchengode.
University	:	**Tamil Nadu Dr. M.G.R Medical University, Chennai- 32.**
Year	:	2016-2017

Aim

This study was aimed to Screen the phytoconstituents and comparative evaluation of antimicrobial, anthelmintic and immunomodulatory activities of *Martynia annua* L. (Martyniaceae) and *Pentanema indicum* (Asteraceae) hydroalcoholic extracts.

Materials and Methods

The qualitative photochemical analysis of *Martynia annua* L. and *Pentanema* indicum screenings were carried out by standard laboratory methods. The *In vitro* antimicrobial activity was evaluated against two gram positives (*E.coli*, *P.aeruginosa)*, two gram negatives (*B.subtilis, S.aureus)* and two fungal cultures (*Candida albicans, Candida krusei)* by disc diffusion method at the concentration level of (50, 100, 150, 200 mg/ml) each extracts compared with standard drugs. MICs and combination antimicrobial effects were evaluated. Followed by extracts of *Martynia annua* L. and *Pentanema indicum* were taken for anthelmintic activity against Indian earthworms *Pheretima posthuma* at the concentrations of 10, 20 & 50

mg/ml. Results were expressed in terms of time for paralysis and time of death of worms. Albentazole (20 mg/ml) was used as reference standard and the combination anthelmintic effect of the plant extracts (1:1) were evaluated. *In vitro* immunomodulatory potential of extracts was assay by evaluate the phagocytosis of *Candida albicans* by a human neutrophil assay.

Results

Preliminary phytochemical screening of hydroalcoholic extracts of *Martynia annua* L. and *Pentanema indicum* showed the presence of alkaloids, saponins, tannins, amino acids, flavonoids, terpenoids, carbohydrates, glycosides, gum and mucilage. Individual extracts shows narrow spectrum of antimicrobial action, but in the combination it shows broad spectrum and potent antibacterial action. Combination only possesses mild antifungal action as compared to the individual extract on fungal species. *Pentanema indicum* and combination have shown more significant anthelmintic action. Both the extracts had shown dose dependent increases in Phagocytosis stimulation, combinations possess more significant immunomodulation action.

Conclusion

Based on the results combination of hydroalcoholic extracts of *Martynia annua* L. and *Pentanema indicum* is much beneficial and nontoxic in unspecified as compared to the synthetic medicines. Further isolation and characterization of phytoconstituents and *In -vivo* evaluations are need to explore this extracts as phytomedicines against pathogenic microorganisms.

CHAPTER-1

INTRODUCTION

CHAPTER - 1

INTRODUCTION

Herbs and medicinal plants are the first medicine, which is a universal phenomenon[1]. Traditional medicine is an important source development of chemotherapeutic agents. The Indian flora offers a variety of plants which is having medicinal properties. These plants can be exploited to find out effective alternative to synthetic drugs. In the developing countries, synthetic drugs are not only expensive and also inadequate for the treatment of diseases but these drugs are cures with adulterations and having many side effects[2].

Medicinal plants are alternative medicines for treatment of various diseases due to their assumed acceptability, effectiveness, affordability, safety and low a cost. Recent days there is increased consumption of herbal formulations by the people because of the strong belief that these products are natural and safe for the treatment of ailments[3].

Indian government and other institutes throughout the world support clinical and laboratory research on herbal medicine within the context of the eastern belief system but herbal medicines isn't widely studied as part of conventional[4].

Attention is mainly focused on the investigation of efficacy of plant based drugs used in the traditional medicine because; they are economical and have little side effects. According to W.H.O about 80% of the world population relies mainly upon herbal remedies.

The WHO has recently defined traditional medicine (including herbal drugs) as comprising therapeutic practices that have been in existence, for hundreds of years, before the development and spread of modern medicine and still in use today[5]. In India, around 20,000 medicinal plants have been recorded however traditional communities are using only 7,000 – 7,500 plants for the treatment of various function diseases[6].

The art of herbal medicine is to dissect pharmacologically and therapeutically active herbal drugs from harmful and toxic plants and to develop combinations of various medicinal plants compounds as safe and efficient herbal remedies. Standardization and strict control measures are necessary to monitor sustainable high quality of herbal products and to exclude contaminations that badly affect patients consuming herbal medicine[7, 8].

Since the dawn of humans, infectious diseases have remained a major cause of death and disability. These days, infectious diseases account for one-third of all deaths in the world; the WHO estimates that nearly 50,000 people die each day throughout the world from infectious diseases. The discovery of antibiotics is one of the novel parts in combating bacterial infections that once ravaged humankind. Different antibiotics exercise their inhibitory activity on different pathogenic organisms. The development and spread of resistance to currently available antibiotics due to the indiscriminate and improper use of current antimicrobial drugs is a worldwide concern. Drug resistance is an increasing global health threat that involves all major microbial pathogens and antimicrobial drugs. At present, clinically important bacteria are not only characterized by single drug resistance, also by multi drug resistance. These are knotty to treat and are responsible for a variety of infectious diseases. In other hand, the pace of development of new antimicrobial agents has slowed down while the prevalence of resistance has grown at high rate. That is, the rate of emergence of antibiotic resistant bacteria is not matched by the rate of development of new antibiotics to combat them[9].

Recently there is increased awareness in the usage natural antimicrobial compounds. The antimicrobial research is geared towards the discovery and development of novel antimicrobial different fails agents[10]. Plant based antimicrobials will help to overcome the resistance problems as well as it will be more reliable than the synthetic products11. A recent ethno-botanical survey of traditional and folk medicine in India has revealed that most of these plants are still

in use by the local tribal people, from ancient time[12]. Helminthiasis is a disease in which a part of the body is infested with parasitic worms like Roundworms (Nematodes), Tapeworms (Cestodes) or Flukes (Trematodes). Although the worms reside in the gastrointestinal tract, sometimes may burrow into the liver and other organs. Since ancient times the medicinal properties of plants have been investigated for scientific advancement throughout the world due to their potent anthelmintic activities[13]. Some broad spectrum anthelmintics (e.g. Piperazine citrate, Albendazole) are effective against parasitic flat worms as well as nematodes. However, majority of drugs are limited in their action (e.g. Praziquantel) as resistance may be developed very quickly[14, 15] and also the toxicity problems may be occurred. Therefore, it is necessary to find out alternative medicine like medicinal plants having broad spectrum anthelmintic activity with less toxicity[16]

The medicinal plants selected for the present study is *Martynia annua* L.*, Pentanema indicum* exhibit the various therapeutic activities are reported. *Martynia annua* L. reported activities are analgesic and antipyretic activity, wound healing activity, antibacterial activity, anthelmintic activity of roots, anticonvulsant activity in albino rats, antioxidant and antifertility activity of leaves, antidiabetic activity of flower and CNS depressant activity performed of root[17]. *Pentanema indicum* fresh roots from the herb are chewed to relieve abdominal pain, Orissa uses the decoction of the roots for relief of fevers in children, *Pentanema Indicum* is used to treat bone fractures among the Paharia in southern Bihar. Thus the *Martynia annua* L. and *Pentanema indicum* plants widely reported and uses as a medicinal plant but different activities in a several region of India[18].

Hence, in tune with this effort, the present study is to screening the combination antimicrobial, anthelmintic and immunomodulatory effect of the *Martynia annua* L. and *Pentanema indicum* in order to understand the nature of the principle component responsible for its medicinal property.

CHAPTER -2

LITERATURE REVIEW

CHAPTER - 2

LITERATURE REVIEW

2.1 ANTI BACTERIAL ACTIVITY

In recent years, drug resistance against human pathogenic bacteria has been widely reported. Because of the side effects and antimicrobial resistance, many scientists have recently paid attention towards biologically active compound isolated from plants.

Natural products can be selected for biological screening based on ethno-medical use of the plants because many infectious diseases are known to be treated with herbal remedies throughout the history of mankind. Even today, plant materials continue to play a major role as therapeutic remedies in many developing countries.

The antimicrobial compounds present in plants may inhibit bacterial growth by many mechanisms. Many studies have identified and isolated the main active ingredients in the plants which are responsible for the antibacterial activity[18].

2.1.1 Natural antibiotics properties of plant secondary metabolites

The plant chemicals are classified as primary and secondary metabolites. Primary metabolites are widely distributed in nature, occurring in one form or another in virtually all organisms. In higher plants such compounds are often concentrated in seeds and vegetative storage organs, which are needed for physiological development particularly in basic cell metabolism. Primary metabolites obtained from higher plants for commercial use as high volume- low value bulk chemicals(e.g. vegetable oils, fatty acids, carbohydrates, etc.).

Plants generally produce many secondary metabolites which are biosynthetically derived from primary metabolites and constitute an important source of antimicrobials, pesticides and many pharmaceutical drugs. From a long period of time medicinal plants or their secondary metabolites have been directly or indirectly

playing an important role in the human society to combat diseases. In contrast to primary metabolites, the secondary metabolites they are synthesized in specialized cell types at distinct stages which, making their extraction and purification difficult. As a result, secondary metabolites that are used commercially as biologically active compounds, are generally high value-low volume products than the primary metabolites (e.g: steroids, quinines, alkaloids, terpinoids and flavonoids) which are commonly used by the drug manufacture in the pharmaceutical industries. These are generally obtained from plant materials by steam distillation or by extraction with organic or aqueous solvents and then molecular weight are generally less than 2000[18].

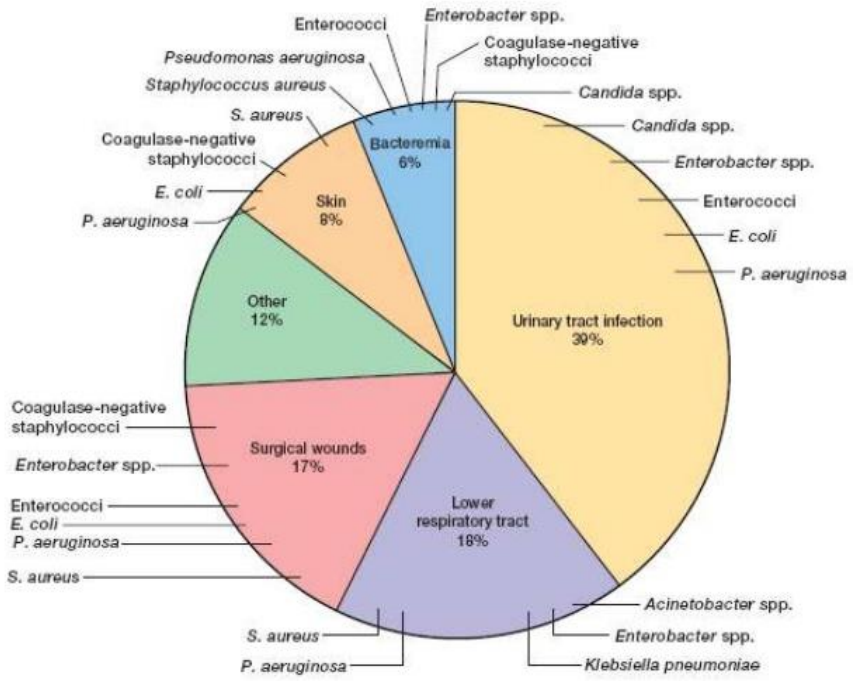


Figure No.1 Epidemiology of Bacterial Organism

Table No: 1 Epidemiology, Therapy, Disease and Resistance of Common causative bacterial organisms[19]

Microorganism	Epidemiology	Infectious disease	Therapy	Resistance
E.coli	Urinary tract infection More common in females than males, tropicals countries is an important cause of childhood diarrhea	UTI, anemia, kidney failure,	Third generation of Cephalosporins	Penicillin, amoxicillin, ciprofloxacin
S. aureus	Human pathogens that Causes wide range of clinical infections.	Impetigo, folliculitis, carbuncle, scalded skin syndrome, osteomylitis, endocarditis	Erythromycin, clindamycin, first generation of cephalosporins	Penicillins, beta lactum antibiotics, Cephalosporin
P. aruginosa	Hospitalized patients (sputum, stools, etc)	Pneumonia, Septic shock, UTI, GI, Skin and Soft tissue infections	Beta lactum antibiotics, aminoglycosides, carbapenems	Cobramycin, Amikacin
B. subtilis	Widely distributed in the environment primary habitat is the soil. These are found in dust, vegetables, water etc.	Food contamination Ocular infection, endocarditis, bacteremia, meningitis	Gentamicin, tetracycline, chloramphenicol, erythromycin, ciprofloxacin, vancomycin	Chloramphenicol, tetracycline, rifampicin streptomycin

2.1.2 Apoorva Pahadia *et al.,* (2013), studied the Antimicrobial activity of hydro alcoholic extract of *Areca catechu.* Disc diffusion method was carried out with amoxicillin as a standard. Hydroalcoholic extract against the fungus at 200 mg/ml concentration. Results suggested that the extract from plant products having synergistic effect hence enhancing the antifungal and antibacterial activity with negligible side effects or toxicity[20].

2.1.3 Sohail *et al.,* (2014), studied that *In vitro* antibacterial activity of *Taraxacum officinale* leaves extracts against different pathogenic strains bacterial. The methanol, chloroform and distilled water extracts was used. Agar well diffusion method was followed. Result showed that distilled water extract had no antibacterial activity and other extracts possess the antibacterial activity against *P. aeruginosa, E.coli, B. subtilis* and *M. luteu.* Minimum Inhibitory Concentration of the extracts against these bacterial strains was in the range of 0.30mg/ml. phytochemical analysis result indicate that the presence of secondary metabolites like alkaloids, tannins and flavonoids might be responsible for antibacterial activity. It could be concluded that extracts of *Tarexacum officinale* have potential is effective against all bacterial pathogenic bacteria[21].

2.1.4 Neethu SK *et al.,* (2016), studied that *In vitro* antibacterial activity and phytochemical analysis of *Gliricidia sepium*(L.) leaf extracts against two gram negative bacteria namely *E.coli* and *P. aeroginosa* by agar cup method. The phytochemical screening was carried out by using the crude leaf extracts in different solvents (water, alcohol and chloroform). Phyto analysis of revealed the presence of glycosides, alkaloids, essential oil, sapponins and flavanoids. The leaf extracts of *G. sepium* were found to have high antibacterial activity. The results suggested that the leaves are a rich source of valuable primary and secondary metabolites exhibiting antimicrobial activity[22].

2.1.5 Mahesh B *et al.,* (2008), evaluated antimicrobial activity of some important medicinal plant against plant and human pathogens. Studied plants were *Acacia nilotica, Sida cordifolia, Tinospora cordifolia, Withania somnifer* and *Ziziphus mauritiana* of Methanolic extracts (root/bark) of these plants showed the significant

antibacterial activity against human pathogens. Result revealed that *A.nilotica* bark and leaf extract showed significant antifungal activity against *A.flavus, Ziziphus mauritiana* and *Tinospora cordifolia* and D.turcica. Methanol extract of *Sida cordifolia* exhibited significant antifungal activity against *F.verticillioides*[23].

2.1.6 Tariro A. Chitemerere *et al.,* (2011), studied the *In vitro* antibacterial activity of selected medicinal plants from zimbabwe. This study was carried out by agar diffusion method MBC, MIC using ampicillin as reference drug. Accumulation of rhodamine 6G in bacteria was used to determine the activity of extracts as drug efflux pump inhibitors. Author concluded that the compounds in these plants can serve as templates for the development of new antibacterial agents as well as efflux pump inhibitors[24].

2.2 ANTI FUNGAL ACTIVITY

Parasitic fungi cause superficial, subcutaneous or deep inside fungal infections in human beings. This is increased drastically in all regions of the world. *Aspergillus niger, A. flavus and Candida albicans* are involved in human infections. These fungi are widely distributed in nature. Aspergillosis and Candidiasis infection in human beings are caused by these fungi.

Candida albicans is an important fungal disease causative that has a worldwide distribution due to the fact that this is frequent opportunistic pathogen in AIDS patients. It is a common commensal of the gastrointestinal and urogenital tracts of human and is also the cause of candiasis in women[25].

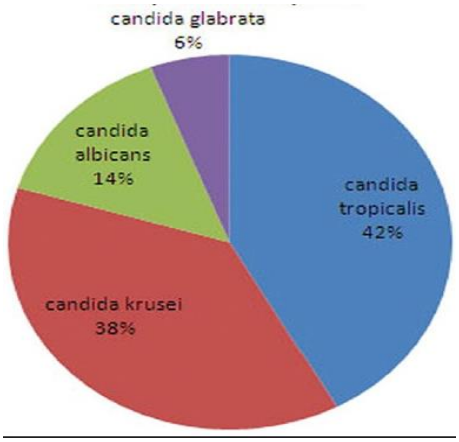


Fig No 2: Candida sp.

Table No. 2 Epidemiology of Fungus[26]

Microorganism	Epidemiology	Infectious disease	Therapy	Resistance
C. albicans	Blood strem infection has been associated with a high mortality rate.	Endocarditis, Esophagitis	Amphotericin B, fluconazole, Cystatin, clotrimaxole	Amphotericin B
C. krusei	Unusual cause of vaginitis	Invasive candidiasis, candidaemia.	amphotericin B, micafungin, caspofungin, anidulafungin	Coriconazoleflu conazole

2.2.1 Hasan M F *et al.,* (2009), studied the antibacterial and antifungal activities of *Polygonum hydroppiper*(L.) root extract carried by disc diffusion method. Root extract possess the antibacterial activity and antifungal activities against various pathogenic organisms. MIC value ranged from 16 to 64 µg/ml. It can be used in the folk medicine in different parts of the world to treat many diseases including bacterial and fungal infections[27.]

2.2.2 Duraipandiyan V *et al.,* (2011) studied the antifungal activity of hexane, ethyl acetate and methanol extracts of 45 medicinal plants they determined minimum inhibitory concentration for each plants against human pathogenic fungi by microdilution method. Concluded that studied plant species possess antifungal properties. Further phytochemical research is needed to identify the active principles responsible for the antifungal activity[28].

2.2.3 Gutal Valyfathulla Shaik *et al.,* (2011), studied aqueous, acetonic, methanolic ethanolic extracts of leaf and stem part of *A.inolucurata* for antifungal activity. Aqueous extracts had no antifungal activity against *A. niger*. Antifungal activity was compared with standard antifungal Co-trimoxazole and fluconazole. The

results obtained in the present study suggested that they can be used in treating diseases caused by the test organisms[29].

2.2.4 Harison M *et al.,* (2014), studied the comparative evaluation of antifungal activity of medicinal plant extracts and chemical fungicides against four plant pathogens. Leaves extract of *Pongamia pinnata*, *Calotropisprocera*, *Neriumindicum*and *Curcuma longa* were taken in aqueous solution and tested against plant pathogenic organisms such as *A.fumigatus, A.solani, Helminthosporium spp.* and *F.solani*. Antifungal activities of various plant extracts were compared with commercially available antibiotics. The antimicrobial potential of the above plant extracts were carried out against the test organism using agar gel diffusion technique. The minimum inhibitory concentration was determined. It was observed that the latex of the plant had a broad spectrum antifungal activity against all the fungi for the tested extracts. These findings supported that traditional uses of the plants in the treatment of different infections[30].

2.3 ANTHELMINTIC ACTIVITY

Helminthiasis is a disease caused by worms such as pinworm, roundworm or tapeworm. Typically, the worms reside in the gastrointestinal tract but may also burrow into the liver and other organs. Infected people excrete helminth eggs in their faces, which contaminate the soil in areas with inadequate sanitation[31]. Other people can then be infected by ingesting eggs or larvae in contaminated food or through penetration of the skin by infective larvae in the soil (hookworms). Parasitic diseases cause severe morbidity and mortality. Filariasis (a cause of elephantiasis), Onchocersiasis (river blindness), and schistosomiasis important parasitic disease of human beings[32]. As per WHO only synthetic drugs are frequently used in the treatment of helminth infestations in human beings but these synthetic drugs are out of reach of millions of people and have a lot of side effect. In view of this, an attempt has been made to study the anthelmintic activity of herbal drug.

2.3.1 The Role of Medicinal Plants as Anthelmintics

Parasitic worms (helminthes) of the gastro intestinal (GI) tract are of major global importance. Over a billion people mainly in developing countries are estimated to be infected with soil transmitted helminthes, whilst helmithes infection is also a serious problem in livestock production worldwide, causing significant e onomic losses and threatening food security[33, 34, 35]. Control of helminthes relies almost exclusively on a limited number of synthetic anthelmintic drugs. The limitations of this reliance on chemotherapy are the threat of parasite developing resistance to drug therapy (already wide spread in animal)[36, 37]. The cost of drugs for small-scale farmers in developing countries against some helminthes, and lack of efficacy of current available drugs[33], therefore need novel and complementary helminthes control options urgently.

The use of natural plant extracts as de-wormers for humans and livestock has long been practiced, however scientific validation of these practices and identification of active compounds has been lacking[38, 39, 40]. Anthelmintic effects of plants are normally ascribed to secondary metabolites such as alkaloids, triterpinoids or polyphenols such as proanthocyanidins[41] also known as condensed tannins (CT). Proanthocyanidins are diverse and widely-occuring group of compounds and consist of polymers of either catechin and / or epicatechin (termed prodelphinidins – PD), with hetero- polymers being common[42]. They are found in plant material from both tropical and temperate areas, and have widely investigated for their antioxidant and anti-inflammatory properties[43,44]. It is also apparent that CT can have anthelmintic effects, reduced worm burdens have been reported in rats administered CT in the diet, or in livestock grazing forages containing CT[45,46]. Moreover, direct anthelmintic effects of purified CT have been confirmed in invitro assays against, amongst others, *Haemonchus Contortus*[47]*, Ostertagia* Ostertagi[48]*, and Ascaris* Suum[49]*.* However, much work remains to be done to establish the spectange of helminthes species that reactivity of CT, i.e. the rare susceptible, and what stages of the cycle are targeted by these molecules.

2.3.2 Kishor K *et al.,* (2014), study was carried out to assess the anthelmintic activity of *Pergularia daemia* leaves. The different extracts of *Pergularia daemia* leaves were evaluated separately on earthworm (Eudrillus eugeniae), round worm (Ascaris lumbricoides) and tapeworm (Taenia solium) using albentazole as standard. The extracts caused paralysis followed by the death worms at tested dose level. The both extracts at the highest tested concentration significantly exhibits ($P<0.01$) paralysis and also caused death of worms as comparable with that of standard drug albentazole. The study displayed that ethanol extract of *Pergularia daemia* leaves shown most significant anthelmintic activity than the aqueous extract.the plant extracts responsible for the activity was suggested[50].

2.3.3 Ashok K *et al.,* (2010), Investigation of anthelmintic activity of three plants from the amarathaceae family. Studied plants are *Amaranthus spinosus, A.caudatus, A.viridis*. They belong to the amaranthaceae family. Three plants using earthworms by investigated the anthelmintic activity. Methanol extracts of the three plants at different concentarations showed dose dependent vermicidal activities. Piperazine citrate was used as reference standard (10mg/ml). Our study found that the plant possess potent anthelmintic activity compared to standard[51].

2.3.4 Saju T *et al*., (2013) studied that photochemical and in vitro anthelmintic study of hydro alcoholic extract of *Costus Pictus* D. Don. The studied phytochemical constituents of the hydro-alcoholic extract of the costus pictus D.DON rhizome and was subjected to preliminary phytochemical analysis. The in vitro anthelmintic activity screening of the extract was performed with earthworm Eudrilus eugeniae. Phytoconstituents possess the study provides a clear evidence for using the hydroalcoholic extract of *Costuspictus* D.DON rhizome in the treatment helminthiasis[52].

2.3.5 Kalyani N *et al.,* (2016), studied that In vitro anthelmintic activity of some medicinel plant Extracts. The aimed at investigate the anthelmintic activity of methanol extract of *Macaranga peltate* stem bark and hydroalcohol extracts of anacardium occidentale leaves and seed testa on African earthworms Eudrilus

Eugenia. Different concentaration of plant three extract possess the anthelmintic activity[53].

2.4 IMMUNE SYSTEM

The immune system is composed of many interdependent cell types that collectively protect the body from bacterial, parasitic, fungal, viral infections and tumor cells. Many of these cell types have specialized functions. The cells of the immune system can engulf bacteria; kill parasites or tumor cells and viral- infected cells. Often, these cells are depending on the T helper cells which subset the activation signals in the form of secretions of cytokines, and lymphokines.

The immune systems mainly devided into two types are

- **Adaptive Immune System**
- **Innate Immune System**

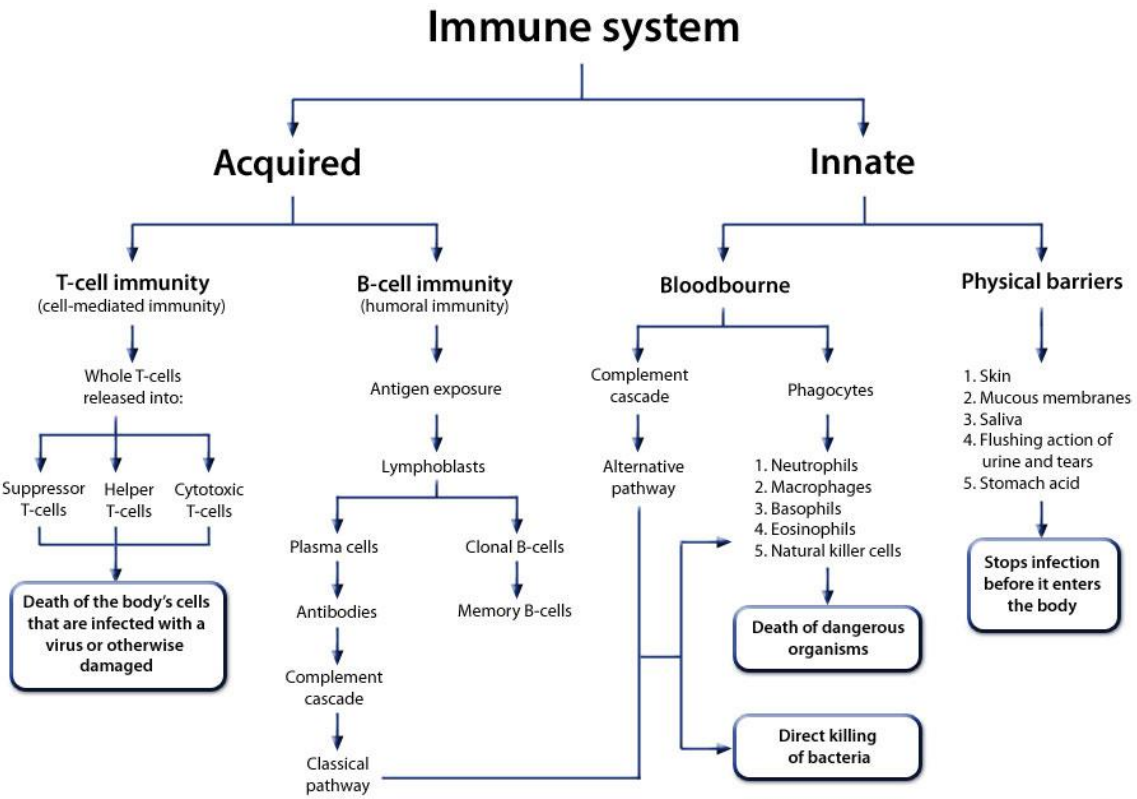


Figure No.3 Immune System

2.4.1 The Innate Immune System

Innate immunity comprises a series of host defenses including physiological barriers cytokines, complement, phagocytes, natural killer (NK) cells, and gamma-delta T cells to provide the initial (nonspecific) response to a pathogen or injury. These responses are phylogenetically ancient and have been developed to cope with pathogens that are encounter regularly but that rarely cause disease. Unlike the adaptive (specific) immune system responses are generic and leave no memory; nonetheless the innate immune system functions effectively to keep organisms healthy. Indeed a failing in innate immunity is hypothesized to contribute to secondary infections in critical illness and death in sepsis[54-58].Stimulation of the active components of the innate immune system occurs by way of Pathogen-associated molecular pattern (PAMP) receptors or Damage – associated molecular pattern (DAMP) receptors. PAMPs are recognized by membrane bound or vesicular Pattern recognition receptors (PRRs) including the Toll-like receptors (TLRs), nucleotide binding oligomerization domain (NOD)- like receptors, and RIG –like receptors. Bacteria stimulate these PRRs to active various intracellular signaling cascades, leading to a proinflammatory response. For example, the gram-negative bacterial endotoxin, lipopolysaccharides, binds to TLR 4, where as the gram – positive peptidoglycan binds to TLR 2 in the setting of tissue damage from an infection or trauma, DAMPs activate the innate immune system through these PRRs.

2.4.2 The Adaptive Immune System

Adaptive or acquired immunity differs from the innate responses as it is specific, has an element of memory, and is unique to vertebrates. The humoral component involves the proliferation of antigen-stimulated B lymphocytes into antibody-secreting plasma cells. The cellular component is mediated by T lymphocytes, the predominant cell types being +helper T cells (Th) and cytotoxic T cells. Recently, regulatory T cells that likely dampen the immune response have been identified. T cells recognize antigens bound to major histocompatibility complex (MHC) proteins by way of T cell receptors that are antigen specific. The lymphocytes

act through secretion of cytokines to elaborate and prime the immune response. This action includes inducing immunoglobulin classes witching of B cells, activation of Tcell and optimization of bactericidal capacity of phagocytes. Th lymphocytes are characterized by expression of CD4 receptor and are activated when MHC type II molecules expressed on professional antigen-presenting cells (dendritic cells, macrophages, and B cells), activate the specific T cell receptor. Th1 cells are regarded as proinflammatory, secreting cytokines such as interferon-G and interkeukin IL-2, stimulate macrophages function and cytotoxic T cell function. Th2 cells have an anti-inflammatory phenotype and secrete cytokine such as IL-4 and IL-10, acting cooperatively to activate B cells. Further, Th cells include the regulatory T cells that act to bampen the immune response and the Th17 class that modulates neutrophil function.A shift from Th1 to Th2 cells has been observed in the later stages of sepsis, possibly induced by the apoptotic cell death of lymphocytes and the subsequent anti-inflammatory phenotype has been associated with secondary infections in these patients[59].

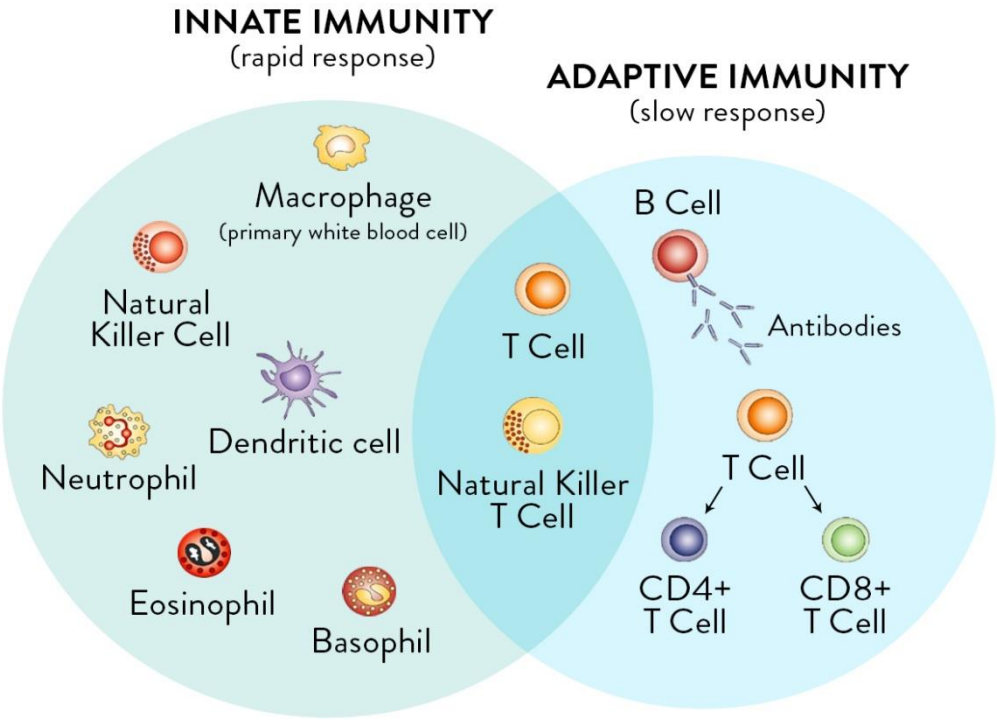


Figure No.4 Types of Immune System

2.4.3 The Organs of the immune system [60-62]

2.4.3.1 Bone Marrow

All the cells of the immune system are initially derived from the bone marrow. They are formed through a process called hematopoiesis. During hematopoiesis, bone marrow-derived stem cells differentiate into either mature cells of the immune system or into precursors of cells that migrate out of the bone marrow to continue their maturation elsewhere. The bone marrow produces B cells, natural killer cells, WBC (agranulocytes) and immature thymocytes, in addition to red blood cells and platelets.

2.4.3.2 Thymus

The function of the thymus is to produce mature T cells. Immature thymocytes, also known as prothymocytes, leave the bone marrow and migrate into the thymus. Through a remarkable maturation process sometimes referred to as thymic education, T cells that are beneficial to the immune system are spared, while those T cells that might evoke a detrimental autoimmune response are eliminated. The mature T cells are then released into the blood stream.

2.4.3.3 Spleen

The spleen is an immunologic filter of the blood. It is made up of B cells, T cells, macrophages, dendritic cells, natural killer cells and red blood cells. This organ can be thought of as an immunological conference center. In the spleen B cells become activated and produce large amounts of antibody. Also, old blood cells are destroyed in the spleen.

2.4.3.4 Lymph nodes

The lymph nodes function as an immunologic filter for the bodily fluid known as lymph. Lymph nodes are found throughout the body, composed mostly of T cells, B cells, dentritic cells and macrophages, the nodes drain fluid from most of our

tissues. Antigens are filtered out of the lymph in the lymph node before returning the lymph to the circulation. In a similar fashion as the spleen, the macrophages and dendritic cells that capture antigens present these foreign materials to T and B cells, consequently initiating an immune response.

2.4.4 The Cell of the Immune System [63-66]

2.4.4.1 T cells

T lymphocyte are usually divided into two major subsets that are functionally and phenotypically (identifiably) different. The T-helper subset, also called the CD4+ T cell, is a pertinent coordinator of immune regulation. The main function of the T helper cell is to augment or potentiate immune responses by the secretion of specialized factors that activate other white blood cells to fight off infection. Another important type of T cell is called the T killer/suppressor subset or CD8+ T cell. These cells are important in directly killing certain tumor cells, viral-infected cells and sometimes parasites. The CD8+ T cells are also important in down-regulation of immune responses. Both types of T cells can be found throughout the body. They often depend on the secondary lymphoid organs (the lymph nodes and spleen) as sites where activation occurs, but they also found in other tissues of the body, most conspicuously in the liver, lung, blood, intestinal and reproductive tracts.

2.4.4.2 Natural Killer cells

Natural killer cells are often referred to as NK cells are similar to the killer T cell subset (CD8+ T cells). They function as effector cells that directly kill certain tumors such as melanomas, lymphomas and viral- infected cells most notably and cytomegalovirus- infected cells. NK cells, unlike the CD8+ (killer) T cells, kill their targets without a prior “conference” in the lymphoid organs. However, NK cells that have been activated by secretions from CD4+ T cells will kill the tumor or viral-infected targets more effectively.

2.4.4.3 B- Cells

The major function of B lymphocytes is the production of antibodies in response to foreign protein of antigen. Antibodies are specialized proteins that specifically recognize and bind to one particular antigenic determinants. Antibody production and binding to a foreign substance or antigen often is critical as a means of signaling other cells to engulf, kill or remove that substance from the body.

2.4.4.4 Granulocytes or Polymorphonuclear (PMN) Leukocytes

Another group of white blood cells is collectively referred to as granulocytes or polymorphonuclear leukocytes(PMNs).Granulocytes are composed of three cell types identified as neutrophils, eosinophils and basophills, based on their staining characteristics with certain dyes. These cells are predominantly important in the removal of bacteria and parasites from the body. They engulf these foreign bodies and degrade them using their powerful enzymes.

2.4.4.5 Macrophages

Macrophages are important in the regulation of immune responses. They are often referred to as scavengers or antigen- presenting cells (APC) because they pick up and ingest foreign materials and present these antigens to other cells of the immune system such as T cell and B cells. This is one of the important first steps in the initiation of immune responses. Stimulated macrophages exhibit increased levels of phagocytic and are also secretory activity.

2.4.4.4 Dendritic cells

Another cell type, addressed only recently, is the dendritic cell. Dendritic cells, which also originate in the bone marrow, function as antigen presenting cells (APC). In fact, the dendritic cells are more efficient APCs than macrophages. These cells are usually found in the structural compartment of the lymphoid organs such as the thymus, lymph nodes and spleen. However, they are also found in the blood stream

and other tissues of the body. It is believed that they capture antigen or bring it to the lymphoid oragns where an immune response is initiated.

2.4 IMMUNOMODULATION:

Immuno modulators are substances that have been shown to modify the immune systems response. They modulate and potentiate the weapons of immune system by keeping them in a highly prepared state for any threat it may encounter. When immune system is in this highly prepared state, the invading organisms do not have the time to build up force and strength before the immune system attacks destroys or weakens the invader. Immunomodulation is the process of modifying an immune response in a positive or negative manner by administration of a drug or compound. Many proteins, amino acids, and natural compounds have shown a significant ability to regulate immune responses, including interferon-´Y (IFN- ´Y) [67-69], steroids[70, 71], DMG[72-75]. These are biological or synthetic substances, which can stimulate, suppress or modulate immune system including both adaptive and innate arms of the immune response. Clinically immunomodulators can be classified into following three catagories[76].

2.5.1 Immunoadjuvants:

These agents are used for enhancing vaccines efficacy and therefore, could be considered specific immune stimulants (eg.Freund adjuvant). The immunoadjuvants hold the promise of being the true modulators of immune response. It has proposed to exhibit them for selecting between cellular and humoral, Th1(helper T1cells) and Th2, (helper T2cells) immunoprotective and immunodestructive and reagents (IgE) versus immunoglobin G (IgG) type of immune responses, which posses to be a real challenges to vaccine designers[77].

2.5.1 Immunostimulants :

These agents are inherently non – specific in nature as they investigated to enhance bodies resistance against infection. They can act through innate immune response and immune response[78]. In healthy individuals the Immunostimulants are

expected to serve as prophylactic and promoter agents i.e. as immunopotentiators by enhancing basic level of immune response and in the individual with impairment of immune response as immunotherapeutic agents[79].

2.5.2 Immunosuppressants :

These are a structurally and functionally heterogeneous group of drugs, which are often concomitantly administered in combination to treat various types of organ transplant rejection and autoimmune disease[80].

2.5.3 Attard E *et al.,* (2009), in vitro immunomodulatory activity of various extracts of Maltese plants from the *Asteraceae* family. Ten plants from were studied for their effects of on human peripheral T-lymphocytes in vitro. Five solvent systems were used to constituents for the plant extract. Tested the one negative BST and five positive BST totally six extract activity was noted. That finally only two extracts caused lymphocyte activation and pronounced blastogenesis similar to that of phytohaemagglutinin (PHA). The results petroleum ether extract of calendula arvensis is relatively non- toxic to peripheral lymphocytes suggesting its potential use as an immune booster[81].

2.5.4 Sheikh Abid A *et al.,* (2013), studied that Invitro immunomodulatory study of different parts of *Prunus cerasus.* Different parts of plant Prunus cerasus L. (sour cherry) were extract with methanol, water-methanol 1:1 and water. Immunomodulatory potential employing NBT, Inos, phagocytosis and Tand B cell proliferation MTT assay. The results of this study demonstrate the immunostimulatory effect of Prunus cerasus L. in a concentration- dependent manner. The results suggest the fruits of sour cherry could be applied as an immunomodulator[82].

2.5.5 Vanitha G K *et al.,*(2016)**,** Evaluation of in vitro immunomodulatory activity of hydroclholic extract of *Ceropegia bulbosa* Roxb.The extract was evaluated at effect of various concentrations (832 µg/ml to 6.5 µg/ml) for secretion of mediators like nitric oxide, superoxide, lysosomal enzyme and myeloperoxidase activity of isolated murine peritoneal macrophages. These performed in the mouse macrophages. In

vitro phagocytic index showed significant results and thus proving he need for confirmation through in vivo studies[83].

2.5.6 Boudoukha *C et al.,* (2015), Immunomodulatory effects of santolina chaemaecyparissus leaf extracts on human neutrophil functions. Aqueous and polyphenolic extracts were prepared from *S.chamaecyparissus* leaves. The elastase release was used as a marker for measuring PMN degranulation, while chemostaxis was performed using a 48-microwell chemotaxis chamber. The phagocytosis and the microbicidal capacity were evaluated using fresh cultures of Candida albicans. This plant may be considered as an interesting source of anti- inflammatory and immunomodulatory aganets[84].

2.5.7 Bhanwase A S *et al.,* (2015), antioxidant and immunomodulatory activity of hydroalcoholic extract and its fractions of leaves of *Ficus benghalensis L.* Hydroalcoholic (FB1) extract and it's four fractions viz. n-hexane (FB2), n-butanol (FB3), chloroform (FB4), and water (FB5) of leaves of *F. benghalensis* investigated for their free radical scavenging activity using 1-1-diphneyl-2-picrylhydrazyl and 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) radicals. A dose-response curve was plotted and IC_{50} values were determined to assess antioxidant activity. Nitroblue tetrazolium test, phagocytosis of killed *Candida albicans* and candidacidal assay were carried out to assess the immunomodulatory activity. Positive non-lymphoid cell number, mean particle number of killed *C. albicans*, percent value of killed *C. albicans* by neutrophils were calculated and presented. All extracts showed antioxidant and prominent immunomodulatory activity with compared to standard. Hydroalcoholic (FB1) extract and its four fractions viz. n-hexane (FB2), n-butanol (FB3), chloroform (FB4), and water (FB5) showed promising antioxidant and immunomodulatory activity[85].

CHAPTER-3

PLANT PROFILE

CHAPTER - 3

PLANTS PROFILE

3.1 *MARTYNIA ANNUA*Linn.

Botanical name	:	*MartyniaAnnua*Linn
Family	:	Martyniaceae

3.1.1 Vernacular names[86]

Tamil	:	Thelkodukkukkay, Puli- nagam
English	:	Devil's claw, Tiger's claw
Hindi	:	Hathajori, Bichu, Ulat-kanta
Malayalam	:	Puli-Nakam
Telugu	:	Garudamukku, Telukondicchhettu
Marahi	:	Vinchu
Gujarati	:	Vichchida
Konkani	:	Shernui

3.1.2 Taxonomical name

Kingdom	:	Plantae
Phyllum	:	Magnoliophyta
Order	:	Scrophulariales
Family	:	Martyniaceae
Genus	:	Martynia
Species	:	MartyniaAnnua

3.1.3 Distribution of MartyniaAnnua

The plant is found in the tropical and sub-tropical region of America, Burma, and West Pakistan. In India it growsalong road sides in abandoned fields and it grows in wastelands, found from coast up to900m.

3.1.4 Descriptions

Herbaceous, stout, erect, branched, clammy pubescent, annual plant growing to a height of 0.25-1 m, covered with dense glandular sticky hairs. The stems are usually woody at base.Leaves are kidney shaped, opposite with lamina reniform, margins entire to shallow-sinuate to be toothed, palmate veined, petiole 9-14cm long and sticky topped glandular hairs present on both the upper and lower leaf blade surfaces.Flowers are bell shaped, purplish white, with dark purple markings. Pedicels 1-2 cm long and recurved in fruit. Corolla is funnel form-campanulate, spotted on the inner surface, the spots yellow, pink or purple. Fruits are hard, bi-lobed, and woody with 2 sharp recurved hooks. Seeds are brown to black, 2 to each pod.

3.1.5 Phytoconstituentsof *MartyniaAnnua*L.

Phytochemical examination of *Martyniaannua*most of the constituents present in the leaves. It indicates the presence of alkaloids, tannins, saponins, glycosides, flavonoids, amino acid steroids and phenols.Flowers also contain cyaniding- 3-galactoside whilst p-hydroxybezoic acid, snapic acid, and gentisic acids are present in leaves and fruits, respectively.

3.1.6 Medicinal uses:

Pharmacological uses of plant *Martyniaannua* analgesic and antipyretic activity, wound healing, antibacterial, anthelmintic activity, anti convulsant, antioxidant etc.was evaluated and reported.

3.1.7Activities reported in*Martyniaannua*L.

3.1.7.1 Ashwani K D *et al.,* (2013), reviewed that*Martyniaannua*L., A Review on Its Ethno botany, Phytochemical and Pharmacological profile. *Martyniaannua*well-known herbaceous plant used in Indian traditional medicines medicines for epilepsy, inflammation and tuberculosis. This weed has possessed the anthelmintic, analgesic, antipyretic, CNS depressant etc. Wide range of chemical constituents present in this plant for example oleic acid, arachidic acid, palmitic acid and etc. aim of this article summarize the uses, pharmacological activity and botany of the *Martyniaannua*L.plant[87].

3.1.7.2 SuryawanshiJS *et al.,* (2013),studied thephytochemicalscreening and antibacterial activity of *Martyniaannua*unripe fruits and flower extracts.Different extract of *martyniaannua*flower and unripe fruits carried out antimicrobial activity. The result was aqueous extract ofunripefruit possess the maximum zone inhibition and flower did not show any recognizable activity. Standard antibiotic used was compared with plant extract. Phytochemical investigation of unripe fruits showed the presence of tannins, saponins and mucilage[88].

3.1.7.3 Kalaichelvi K *et al.,* (2016), studied that phytochemical screening and antibacterial activity of leaf extract of *Martyniaannua,L.* and *Premnalatifolia*,Roxb. The present study was phytochemical screening and antimicrobial activity of different extracts (aqueous, ethanol, acetone and petroleum ether) carried out by phytochemical screening assay and disc diffusion method. A finally ethanolicleaves extract was possess better activity compared with another solvents of above plants leaves extract. These results suggest that the Phytoconstituents but only aminoacids absent the all solvents of*Martyniaannua*and *Premnalatifolia* leaves extracts and stable antimicrobial activity, to establish the scientific base for modern medicine[89].

3.1. 7.4 Padmapriya M *et al.,* (2016), studied the phytochemical screening study of *Martyniaannua*. The study was nature has been a good source medicinal agent for many years and modern drugs based on their use in traditional medicine. Phytochemicals nutrientsare referred to in the plant and high potential cure many diseases.The present study was different solvents extracts (ethanol,methanol and chloroform). Phytoconstituents present in the various part of this plant (stem, leaf, flower) which have proved that the plants are known for its therapeutic value[90].

3.1.7.5 Aziz – ur- Rehman*et al.,* (2012), studied that*Martyniaannua:* Comparative Antioxidant Potential of Its Stem and Leaves. Thisstudy investigatesthe antioxidant and scavenging activity of various fractions of stem and leaves of *martyniaannua*L. The finally concluded that n-butanol soluble fraction of stem contains many antioxidant compounds. Similarly, the ethyl acetate soluble fraction of leaves is also a valuble source of antioxidants[91].

Figure No.5*Martyniaannua L.* plant

Figure No.6*Martyniaannua* L. seeds

3.2 *PENTANEMA INDICUM*[92]

Botanical name	:	*Pentanemaindicum*
Synonym	:	*Inulaindica, Vicoaindica*
Family	:	*Asteraceae*

3.2.1 Vernacular name:

Gujarati	:	SonaSali
Marathi	:	Sonkadi
Hindi	:	Bichhloo
Kannada	:	Mugutisoppu
Tamil	:	Mookuti and Poondu

3.2.2 Plant Description:

It is an erect herb with reddish puberulous stems and stems are branched in the upper part. Branches are cylindrical and leafy. Leaves are ovate- lanceolate in shape and it'scordate, auriculate and sessile. 3-8 cm long and hairy. In this Indian medicinal plant, the flowers heads are yellow in colour and it available throughout the year.

3.2.3 Medicinal values of Plantparts:

Fresh roots from the herb are chewed to relieve abdominal pain. In this plants are used to relieve scorption stings. The inflorescence is used for treating throat disorders. Pentanemaindicum is used in the form of a decoction by women as an oral contraceptive. It is used to treat bone fractures among the paharia in southern bihar. Thus, the pentanemaindicumplant is widely used as a medicinal plant but in different method in several regions india.

FigureNo. 7*Pentanemaindicum*

CHAPTER-4

AIM AND OBJECTIVE

CHAPTER - 4

AIM AND OBJECTIVE

The revival of interest in herbal therapy recently has been witnessed in many countries and use of herbal drugs is increased because of its potency and low toxicity. Now a days in the modern advance stage scientific method of evaluation using phytochemical investigation to isolate the components present, pharmacological and microbiological screening for their therapeutic efficacy have tend to rational usage of the medicinal plants.

According to the literature, the different parts of the *Martynia annua* L. and *Pentanema indicum* wiz, leaf, root, seed, stem and whole plant have been used for various medicinal purposes like analgesic, anti- inflammatory, wound healing, anti-oxidant, antibacterial, stomach ache, hypertension, anti diabetic etc.

In this direction, our effort was devoted in identifying preliminary Phytoconstituents of *Martynia annua* L. and *Pentanema indicum* and to investigate the comparative *In vitro* anti microbial, anthelmintic, immunomodulatory activities of hydroalcoholic extract of *Martynia annua* L. and *Pentanema indicum*.

CHAPTER- 5

PLAN OF WORK

CHAPTER- 5

PLAN OF WORK

1. Collection and authentication of plant material
2. Preparation of plant extracts
    a) Hydro alcoholic extraction *of Martynia annua* L.
    b) Hydro alcoholic extraction *of Pentanema indicum*
3. Preliminary phytochemical screening
    a) Tests for carbohydrates
    b) Test for glycosides
    c) Test for alkaloids
    d) Test for proteins and free amino acids
    e) Test for phenolic compound and tannins
    f) Test for flavonoids
    g) Tests for fixed oils and fats
    h) Tests for steroids and triterpenoids
    i) Test for saponins
    j) Test for mucilages and gums
4. Antibacterial activity
    a) Antibacterial activity by Agar disc diffusion method
    b) Minimum Inhibitory Concentration (MIC)
    c) Synergistic Activity
5. Anti Fungal Activity
    a) Minimum Inhibitory Concentration
    b) Synergistic activity
6. Anthelmintic activity
7. Immunomodulatory Response
    a) Phagocytosis of *Candida albicans* assay
8. Statistical analysis

CHAPTER-6

MATERIALS AND METHODS

CHAPTER - 6

MATERIALS AND METHODS

6.1 COLLECTION AND AUTHENTICATION OF PLANT MATERIAL

The whole plants of *Martynia annua* L. and *Pentanema indicum* were collected from in and around the region of Chitharampoondi, Erode, Tamil Nadu in the month of January 2017. The plant materials were identified and authenticated by **Dr. R. Prabakaran.,** M.sc, Ph.D, Head, Department of Botany, Vivekanandha College of Arts and Science, Tiruchengode. A voucher specimen was submitted and deposited in the Department of pharmacology, Swamy Vivekanandha College of Pharmacy, Tiruchengode, Tamil Nadu.

6.2 PREPARATION OF PLANT EXTRACTS

The Freshly collected whole plants of *Martynia annua* L. and *Pentanema indicum* were shade dried at room temperature. The dried plant materials were subjected to size reduction to a coarse powder by using dry grinder and passed through sieve no. 40 was used for extraction.

6.2.1 Hydro alcoholic extraction

Powdered material of *Martynia annua L.* (100gm) was extracted by cold maceration with 1000 ml of hydro alcoholic (8:2) for 48 hrs. The extract was filtered by whatman no.1 filter and the filtrate was evaporated under reduced pressure using rotatory vaccum evaporator until all solvent was removed to give a dark coloured molten extract. The percentage yield of the hydro alcoholic extract *Martynia annua* L. was 8.06 %w/w. The extract was stored in airtight containers in refrigerator maintained below 10 ^{0}C until further use.

Powdered material of *Pentanema indicum* (100gm) was kept for cold maceration with 1000 ml of hydro alcoholic (7:3) for 48hrs. The extract was filtered by whatman no.1 filter and the filtrate was evaporated under reduced pressure using

rotatory vaccum evaporator until all solvent was removed to give a dark coloured molten extract. The percentage yield of the hydro alcoholic extract *Pentanema indicum* was 8.9 %w/w. The extract was stored in airtight containers in refrigerator maintained below 10 ^{0}C until further use.

6.3 PRELIMINARY PHYTOCHEMICAL SCREENING [93-98]

The hydroalcoholic extracts of *Martynia annua* L. and *Pentanema indicum* was subjected to following preliminary phytochemical screening for the presence or absence of phytoconstituents by the following methods.

6.3.1 Tests for carbohydrates

A small quantity of the extract was dissolved in 4 ml of distilled water and filtered. The filtrate was subjected to different tests to detect the presence of carbohydrates.

6.3.1.1 Molisch' test

Filtrate was treated with 2-3 drops of 1% alcoholic α – naphthol solution and 2 ml of conc. Sulphuric acid was added along the sides of the test tube. Appearance of brown ring at the junction of two liquids shows the presence of carbohydrates.

6.3.1.2 Fehling's test

A small portion of the filtrate was treated with fehling's solution 1&2 and then heated on a water bath. After the incubation period, a brick red coloured precipitate was formed, which indicates the presence of carbohydrates.

6.3.1.3 Barfoed 's test

A small portion of the filtrate was treated with barfoed's reagent. A red colored precipitated was formed, which indicates the presence of carbohydrates.

6.3.1.4 Benedict's test

The filtrate was treated with equal volume of benedict's reagent and heated for space 5mins in water bath. The solution appears green, yellow or red color may indicate the presence of reducing sugar.

6.3.1.5 Selliwanoff's test

To 1 ml of filtrate add 3ml of selliwanoff's reagent. This solution was heated in water bath for 1-2 minutes. Appearance of red colour indicates the presence of reducing sugars.

6.3.2 Test for glycosides

Another portion of the extract was hydrolysed with hydrochloric acid for few hours on a water bath and then hydrolysate was subjected to following tests to detect the presence of different glycosides.

6.3.2.1 Legal's test

To the hydrolyse 1ml of pyridine and few drops of sodium nitroprusside solutions were added and then it was made alkaline with sodium hydroxide solution. Appearance of pink to red colored shows the presence of glycosides.

6.3.2.2 Borntrager's test

Hydrolysate was treated with chloroform and then the chloform layer was separated. To this equal quantity of diluted ammonia solution was added. Ammonia layer acquires pink color, showed the presence of glycosides.

6.3.2.3 Baljet's test

The hydrolysate was treated with sodium picrate solution. A yellowish orange colored was formed, which indicated the presence of glycosides.

6.3.2.4 Keller-killiani test

To 2ml of filtrate, add 1 ml of GAA and 1drop of 5% $Fecl_3$ shake well and add few drops of conc.H_2SO_4 through the sides of the test tube. A reddish –brown color

appears at the junction of the two liquid layer, upper layer appeared as bluish-green. It indicated the presence of glycosides.

6.3.2.5 Conc. H_2SO_4 test

To the filtrate, add 1m l of conc.H_2SO_4 and allow standing for 2 mins. A red precipitate was formed, which indicated the presence of glycosides.

6.3.3 Test for alkaloids

A small portion of the extracts were stirred separately with few drops of dil. Hcl and filtered. The filtrate was tested with various reagents for the presence of alkaloids. The reagents are:

1. Dragendroff's reagent - Reddish brown / orange brown precipitate
2. Mayer's reagent - Creamy white color precipitate
3. Wagner's reagent - brown color precipitate
4. Hager's reagent - yellow precipitate

6.3.4 Test for proteins and free amino acids

A small quantity of the extracts were dissolved in few ml of water and treated with following reagents.

6.3.4.1 Million's reagent

Appearance of red color showed the presence of protein and free amino acid.

6.3.4.2 Ninhydrin reagent

Appearance of purple colored shows the presence of proteins and free amino acids.

6.3.4.3 Biuret's test

Equal volume of 5% sodium hydroxide solution and 1 % copper sulphate solution was added. Appearance of pink or purple color showed the presence of proteins and amino acids.

6.3.4.4 Xantheoproteic test

To 2 ml of filtrate, add 3 mL of conc.H_2SO_4 solution through the test tube. A white precipitates was formed, which indicated the presence of proteins.

6.3.5 Test for phenolic compound and tannins

The extracts were diluted separately with distilled water and filtered. The filtrate was treated with following procedures.

6.3.5.1 Ferric chloride test

The filtrate was treated with 5% $FeCl_3$ solution. A violet precipitate was formed which indicated the presence of phenolic compounds and tannins.

6.3.5.2 Lead acetate test

Few ml of filtrate was treated with lead acetate solution, a white precipitate was formed which indicateed the presence of phenolic compounds and tannins.

6.3.5.3 Ellagic acid test

To 2 ml of filtrate, add 5% GAA and 5%$NaNO_2$ solution. A brown precipitate is formed, which indicateed the presence of tannins / phenolic compounds.

6.3.6 Test for flavonoids

6.3.6.1 Alkaline reagent test

The extract was treated with aqueous sodium hydroxide solution – Blue to violet colored (anthocyanins) yellow colored (flavones), yellow to orange (flavonones).

6.3.6.2 Shinoda's test

Small quantities of the extract were dissolved in alcohol, to them piece of magnesium followed by Con.hydrochloric acid drop wise added and heated. Appearance of magenta color showed the presence of flavonoids.

6.3.6.3 Ferric chloride test

To a small quantity of alcoholic solution of extract few drops of neutral ferric chloride solution was added. Blackish red color was observed, showing the presence of flavonoids.

6.3.6.4 Fluorescence test

Alcoholic solution was seen under UV-light. Green fluorescence was observed, indicated the presence of flavonoids.

6.3.6.5 Conc.H_2SO_4 test

To a small portion of filtrate, add few drops of Conc.H_2SO_4 solution through the sides of the test tubes. A yellowish-orange precipitate was formed, which indicated the presence of flavonoids.

6.3.7 Tests for fixed oils and fats

6.3.7.1 Spot test

Small quantities of various extract were separately pressed between two filter papers. Appearance of oil stain on the paper indicated the presence of fixed oil.

6.3.7.2 Saponification test

Few drops of 0.5N alcoholic potassium hydroxide were added to small quantity of various extracts along with a drop of phenolphthalein, the mixture was heated on a water bath for 1-2 hours, formation of soap or partial neutralization of alkali indicated the presence of fixed oils and fats.

6.3.8 Tests for steroids and triterpinoids

A small quantity of extracts was dissolved in 5ml of $CHCl_3$ separately and the solution was subjected to the following tests:

6.3.8.1 Libermann – burchard test

The extract treated with few drops of acetic anhydride, boil and cool. Then add con. Sulphuric acid from the side of test tube, brown ring is formed at the junction two layers and upper layer turns green which shows presence of steroids and formation of deep red indicated presence of triterpenoids.

6.3.8.2 Salkowski test

Treated the extract with few drop of conc. Sulphuric acid, red color at lower layer indicates presence of steroids and formation of yellow coloured lower layer indicated presence of triterpenoids.

6.3.9 Test for saponins

The extract was diluted with 20ml of distilled water and it was agitated on a graduated cylinder for 15 minutes. The presence of saponins was indicated by formation of layer of foam.

6.3.10 Test for mucilages and gums

Small quantities of extract were added separately to 25 ml of absolute alcohol with constant stirring and filtered. The precipitates was dried in oil and examined for its swelling property for the presence of gum and mucilage.

The phytoconstituents present in the hydroalcoholic extract of *Martynia annua* L. and *Pentanema indicum* were shown in table no -1.

6.4 ANTIBACTERIAL ACTIVITY

6.4.1 Test Microorganisms

Four pathogenic bacterial strains were used as the test organism for antibacterial screening. Among them two gram positive organism were *Bacillus subtilis* and *Staphylococcus aureus* and two gram negative organism were *Escherichia coli* and *Pseudomonas aeruginosa* were collected from Department of Microbiology, Vivekanandha College of Arts and Science, Tiruchengode.

6.4.2 Preparation of the inoculum[99]

To prepare the bacterial inoculum a loopful of test microorganism was taken and subsequently sub-cultured into separate test tube containing the nutrient agar broth. Then the tub was subject to incubation for 24 h at 37^0C, the obtained broth with microorganism was standardized to have a uniform population density of microorganisms.

6.4.3 Antibacterial activity by Agar disc diffusion method

The antibacterial activity of the hydroalcoholic extracts of *Martynia annua* L. and *Pentanema indicum* were determined by disc diffusion method described by Kirby Bauer minor modification[100]. Mueller-Hinton agar media was used to prepare the test plate. In all the cases, 100 µl of the test bacteria were grown in 10 ml of fresh media until they reached a count of approximately 10^8 cells/ml of bacteria and then 100 µl microbial suspension was spread onto the nutrient agar plates.

Different concentrations of 50, 100, 150 and 200 mg/ml of hydroalcoholic extracts were prepared with 1% Dimethyl sulfoxide solution. The extracts were tested by using 5 mm sterilize filter paper discs. Later there discs were impregnated with 25 µl of test samples (hydroalcoholic extracts of *Martynia annua* L. and

Pentanema indicum) allowed to dry and placed aseptically on sensitivity plates with appropriate antibacterial standard. The plates were incubated for 24 hrs at 37^0C for bacteria[101]. Standard antibiotics Bacitracin and Cefotaxime were used as positive control. The positive results (sensitivity) were established by the presence of clear zone of inhibition around active extracts which were measured with a meter rule and diameters were recorded based on millimetres.

6.4.4 Minimum Inhibitory Concentration (MIC)

6.4.4.1 Preparation of bacterial solution

A single colony of bacteria was incubated overnight at 37^0C. The bacterial suspension was centrifuged at 4000 rpm for 5 mins. The supernatant was poured off and bacteria were resuspended in normal saline. The above centrifugation and resuspension was repeated. After the last run, the bacteria were resuspended in 20 mL of normal saline (0.9 % NaCl w/v). Using UV spectrophotometer (of wave length 500 nm) and graphs of viability for the particular strain, the degree of dilution necessary to obtain a final concentration of 5×10^6 cfu/mL was calculated and the bacterial suspension was prepared accordingly.

6.4.4.2 Dilution Procedure for Minimum Inhibitory Concentration

Minimum inhibition concentration was done to determine the lowest concentration of extract, where it can show the bactericidal and bacteriostatic effect. The test was performed in 96 well microtitre plates. Microtitre plate wells from each column in row 1 were marked and 100µl (500 mg/ml) of stock solution (hydroalcoholic extracts of *Martynia annua* L. and *Pentanema indicum*) was added. 50µl of sterile ddw was added to rows 2-12. Two fold serial dilutions were performed by transferring 50µl of solution from row 1 to 2, using a multichannel pipette. This was repeated down the row 2 to 12. 40 µl of double strength nutrient broth and 10 µl of bacterial culture was added to all the wells in separate column, so the final concentration of the inoculums in all wells. To prevent dehydration, the plates were covered with plastic cover and then incubated at 37^0C for overnight. The bacterial growth was determined after addition of 40µl of tetrazolium red (0.2mg/ml). The minimum inhibitory concentrations (MIC) of the isolates were taken as the lowest

concentration of the extract of which the bacterial tested did not show visible growth[102].

6.4.5 Synergistic Activity

The synergistic antibacterial activity of each plant extracts determined by disc diffusion method with slight modificatio[103,104]. Mueller- Hinton Agar was the media which is selected for preparing test plates. Broth culture of test bacteria was (100 µl) spread on the agar media in petriplates. And 25 µl minimum inhibitory concentration of the test samples at 1:1 ration was impregnated on 5mm sterilized filter paper discs and then allowed to dry for few minutes at room temperature was aseptically placed on plates. The plates were incubated at 37^0C about 24 hrs. The diameters of the inhibition zones were measured in millimeters after 24 hrs.

6.5 Anti Fungal Activity

6.5.1 Test organism

The fungal organisms used for susceptibility testing were *Candida albicans* and *Candida krusei* were collected from Department of Microbiology, Vivekanandha College of arts and science.

6.5.2 Procedure

The assay was performed by agar disc diffusion method. Active cultures for the experiment were prepared by transferring a loop full of culture was from the stock cultures into the test tubes containing 5 mL of Sabouraud Dextrose broth, which were incubated at 48 hrs at 37^0C. Antifungal activity of the plant extract was determined by disc diffusion method on Sabouraud Dextrose agar medium (SDA). SDA medium is poured in to the pertiplate. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistened with the fungal suspension (10µl). The concentrations of (50, 100, 150, 200 µg/ml) disc were placed in SDA plates. The plates were incubated at 37^0C for 7 days. Then antifungal activity of the plant extract was determined by measuring the diameter of the inhibitory zones using a scale and compared with those produced by the standard Itraconazole. The diameter of zones of inhibition less than 5 mm were considered as insignificant[105].

6.5.3 Minimum Inhibitory Concentration

6.5.3.1 Preparation of Inoculum

Organisms were subcultured on nutrient agar, followed by incubation for 24 h at 37°C. Inoculum was prepared by transferring several colonies of *Candida albicans* and *Candida krusei* to sterile nutrient broth[refer sir]. The suspensions were mixed for 15 s and incubated for 24 h at 37°C. Required volume of suspension culture was diluted to match the turbidity of 0.5 Mc Farland standard (1.5 × 108 CFU/mL). Minimum inhibitory concentration (MIC) was considered the lowest concentration of the sample that prevented visible growth.

6.5.3.2 Broth Dilution Assay

A series of 15 tubes were filled with 0.5 ml of sterilized nutrient broth. Sequentially, test tubes 2–14 received an additional 0.5 ml of the sample serially diluted to create a concentration sequence from 500 to 0.06 µg. The first tube served as a control. All the tubes received 0.5 ml of inoculum. The tubes were vortexed well and incubated for 24 h at 37°C. The resulting turbidity was observed, and after 24 h MIC was determined to be where growth was no longer visible by assessment of turbidity by optical density readings at 600 nm.

6.5.3.3 Synergistic activity

Synergistic antifungal activity of hydroalcoholic extracts of *Martynia annua* L. and *Pentanema indicum* was determined by disc diffusion method on Sabouraud Dextrose agar medium (SDA). After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistened with the fungal suspension (10µl). The minimum inhibitory concentration of the test samples at 1:1 ration was impregnated disc were placed in SDA plates. The plates were incubated at 37^0C for 7 days. Then antifungal activity of the plant extract was determined by measuring the diameter of the inhibitory zones with a meter rule and diameters were recorded based on millimetres [107].

6.6 ANTHELMINTIC ACTIVITY

6.6.1 Experimental worms

Anthelmintic activity was carried out in Indian adult earthworms (*Pheretima posthuma)* due to its anatomical resemblance with the intestinal roundworms parasites of human beings. They were collected from moist soil and washed with normal saline to remove all fecal matters.

6.6.2 Procedure

6.6.2.1 Administration of albentazole

Albentazole (20mg/ml) was prepared by using 1%w/v of Carboxy Methyl Cellulose was as a suspending agent as administered as per method of extract. Used as a standard for anthelmintic activity.

6.6.2.2 Administration of extract

The suspension of hydroalcoholic extract of *Martynia annua* L. and *Pentanema indicum* at different concentration (10, 20, 50 mg/ml) were prepared by using 0.5 w/v of CMC as a suspending agent and final volume was made upto 20 ml for respective concentration. Albendazole was used as standard. Groups of approximately equal size (5-6 cm), weight 0.32-0.50 gm worms consisting of three individual earthworms individually in each group were released into in each 20 mL of desired concentration of drug and extracts in the petridish[108].

6.6.3 Experimental Design

Group I	**:**	Normal saline (0.9% $NaCl_2$
Group II	**:**	Albentazole (20 mg/ml)
Group III	**:**	HAMA (10 mg/ml)
Group IV	**:**	HAMA (20 mg/ml)
Group V	**:**	HAMA (50 mg/ml)
Group VI	**:**	HAPI (10 mg/ml)
Group VII	**:**	HAPI (20 mg/ml)
Group VIII	**:**	HAPI (20 mg/ml)
Group IX	**:**	HAMA (50 mg/ml) + HAPI (50 mg/ml)

Mean time for paralysis was noted when no movement of any sort could be observed, except when the worm was shaken vigorously; The time death of worm

(min) was recorded after ascertaining that worms neither moved when shaken not when given external stimuli[109].

6.7. Immunomodulatory Response[110]

6.7.1 Phagocytosis of candida *albicans* assay

Phagocytosis of *candida albicans* test was carried out according by ponkshe & Indap, 2002.

6.7.2 Media preparation

Sabouraud broth was prepared for the present work by dissolving 30 gm sabouraud broth (containing peptone 10 g/L and dextrose 20 g/L) (Himedia) in 1000ml of distilled water. The broth was further sterilized at 121^0 c for 15 min at 15 lbs pressure and was used according to the protocol.

Reagents and their preparation:

Hank's balanced salt solution:	(g/L of distilled water)
Calcium chloride	0.14
Potassium chloride	0.40
Potassium phosphate monobasic	0.06
Magnesium chloride	0.10
Magnesium sulphate	0.10
Sodium chloride	8.00
Sodium bicarbonate	035
Sodium phosphate dibasic	0.048
Dextrose	1.00

The solution was sterilized at 121^0 C for 15 min at 15 lbs pressure and was added with 0.01g of phenol red (HiMedia).

Giemsa stain (HiMedia): *Giemsa stain* for the present study was prepared by adding 0.67 mL of *Giemsa stain* (HiMedia – S011) in 30 mL distilled water.

6.7.3 Culture preparation

Sabourauds broth was inoculated with *Candida albicans* (MTCC 183) and was incubated overnight. The overnight culture of *Candida albicans* was centrifuged to get the pellet and supernatant was removed. The collected cell pellet was washed

with Hank's balanced salt solution and centrifuged again. This was repeated for 3-4 times and the final cell pellet was mixed with a mixture of sterile Hank's balanced salt solution and human serum in proportion 4:1. The concentration of 1 X 10^8 cells was used in present work.

6.7.4 Phagocytosis evaluation

Briefly, by using finger prick method human blood was added onto the clean glass slide and was incubated at 37^0 C for 25 min for clotting. Normal sterile was further used to remove the clot, in such a way not to wash the adhered neutrophils. 100 µl of different concentration (5 mg/ml and 10 mg/ml) of test plant extracts were added on to adhered neutrophils and was incubated at 37^0 C for 15 mins. This was followed by addition of predetermined concentration of *Candida albicans* suspension and incubated at 37^0 C for 1 h. The slides were then drained, fixed using methanol and were stained with giemsa stain.

The number of *Candida albicans* cells phagocytosed by a human neutrophil on the slide was determined microscopically using morphological criteria. The number of candida cells phagcide/engulfed by a neutrophil was taken as phagocytic index (PI).

6.7.5 Determination of phagocytosis stimulation/ immunostimulation

Immunostmulaton was calculated in percentage using the following equation.

Immunostimulation % = PI (samples) – PI (control)/ PI (control) *100.

Where, PI of samples: Phagocytic index of the plant extracts, PI of control: phagocytic index without the test plant extracts (i.e. normally by neutrophils).

6.8 Statistical analysis

Data presented as mean ± standard error mean (SEM) and were analyzed by One-way analysis of variance (ANOVA) (SPSS for windows, version 18.0). The values were considered significantly different at $p<0.05$.

CHAPTER-7

RESULT

CHAPTER - 7

RESULTS

7.1 Preliminary Phytochemical screening of hydroalcoholic extracts of *Martynia annua* L. and *Pentanema indicum*

In this study hydro alcoholic extracts of both plants were subjected to phytochemical screening and the results are represented in Table-1.

Alkaloids, Carbohydrates, Flavonoids, Saponins, Steroids, Mucilages were present in both the plant extracts. Glycosides are absent in *Martynia annuna* L. extract. Protein and fixed oil are absent in the both the Hydroalcoholic extracts. Phenol constituent was absent in the *Pentanema indicum*. Presence of Alkaloids, Flavonoids, amino acids, saponins, carbohydrate, Tannins, etc are commonly present in the both the extracts which may be responsible for Antimicrobial activity.

7.2 Antibacterial activity of hydroalcoholic extracts of *Martynia annua* L. and *Pentanema indicum*

Table 2 & Figure 8, 9, 10,11 shows the anti bacterial activity of the hydro alcoholic plant extracts of *Martynia annua L. and Pentanema indicum* were tested against the bacterial species *E. coli, P. aruginosa, B. subtilis, S. aureus*. The standard drugs used are cefotaxime (10mg/ml) & Bacitracin (10 µg/ml).

The HAEMA and HAEPI produce dose dependent increase in zone of inhibition. HAEMA shows greater zone of inhibition in *E.Coli, P.aruginosa* (G- organism) as compare to the G+ organisms. HAEPI shows greater zone of inhibition in *B.subtilis, S.aureus* (G+ organism) as compare to the G- organisms. More over HAEMA and HAEPI individually does not show greater response as compare to the Standards cefotaxime (10mg/ml) & Bacitracin (10 µg/ml).

Table 3 : Preliminary Phytochemical screening of hydroalcoholic extracts of *Martynia annua* L. and *Pentanema indicum*

S.No.	Plant Constituents	Identification tests	HAEMA (8:2)	HAEPI (7:3)
1.	Alkaloids	Mayer's test	-	-
		Hager's test	+	+
		Wager's test	-	+
		Dragendroff's test	-	-
2.	Carbohydrates	Molisch's test	+	+
		Fegling's test	+	+
		Benedict's test	-	-
		Barfoed's test	+	-
		Selwinoff's test	+	+
3.	Glycosides	Borntrager's test	-	+
		Legal's test	-	-
		Keller killiani test	-	+
		Conc.H_2SO_4	-	-
4.	Phenolic compounds & Tannins	Ferric chloride test	+	-
		Lead acetate test	+	-
		Ellagic acid test	-	-
5.	Protein and amino acids	Million's test	-	-
		Ninhydrin test	-	-
		Biuret test	-	-
		Xanthoprotein test	-	-
6.	Saponins	Foam test	+	+
7.	Steroids & triterpinoids	Libermann- burchard test	+	+
		Salkowski test	-	-
8.	Fixed oils & fats	Spot test	-	-
		Saponification test	-	-
9.	Flavonoids	Shinoda test	+	+
		Alkaline reagent test	-	-
		Conc.H_2SO_4	+	+
		Ferric chloride test	-	-
		Fluorescence test	+	+
10.	Mucilage & gums	With 90% alcohol	+	+

Table 4 : Antibacterial activity of hydroalcoholic extracts of *Martynia annua* L. and *Pentanema indicum*

Bacterial Species	Zone of inhibition in mm									
	HAEMA (mg/ml)				HAEPI (mg/ml)				Standard	
	50	100	150	200	50	100	150	200	Cefotaxime (10 µg/ml)	Bacitracin (10 µg/ml)
E.coli	6	8	9.1	10.7	5.2	5.9	6.5	7.4	10	-
Pseudomonas sp.	7.4	8	10	11	5.5	6	7	9	11.8	-
B. subtilis	5.5	6	6.9	9.5	5.2	7.5	10	11	-	12
S. aureus	5.2	6	7.5	9	5	7	7.5	10	-	12

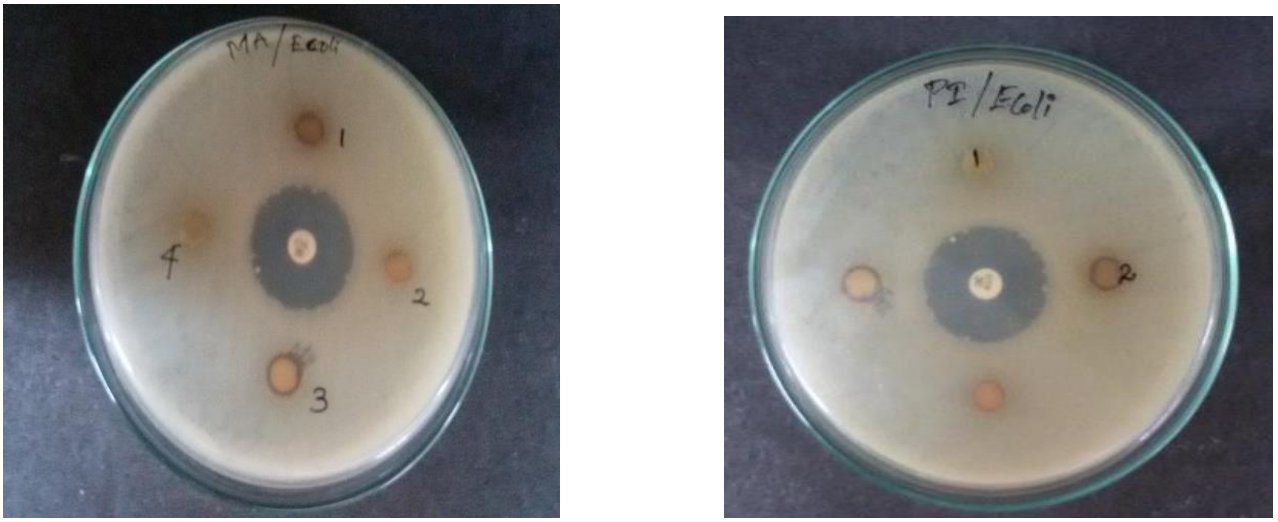


Figure No. 8 Disc diffusion plate showing zone of inhibition of HAEMA & HAEPI against *E. coli*.

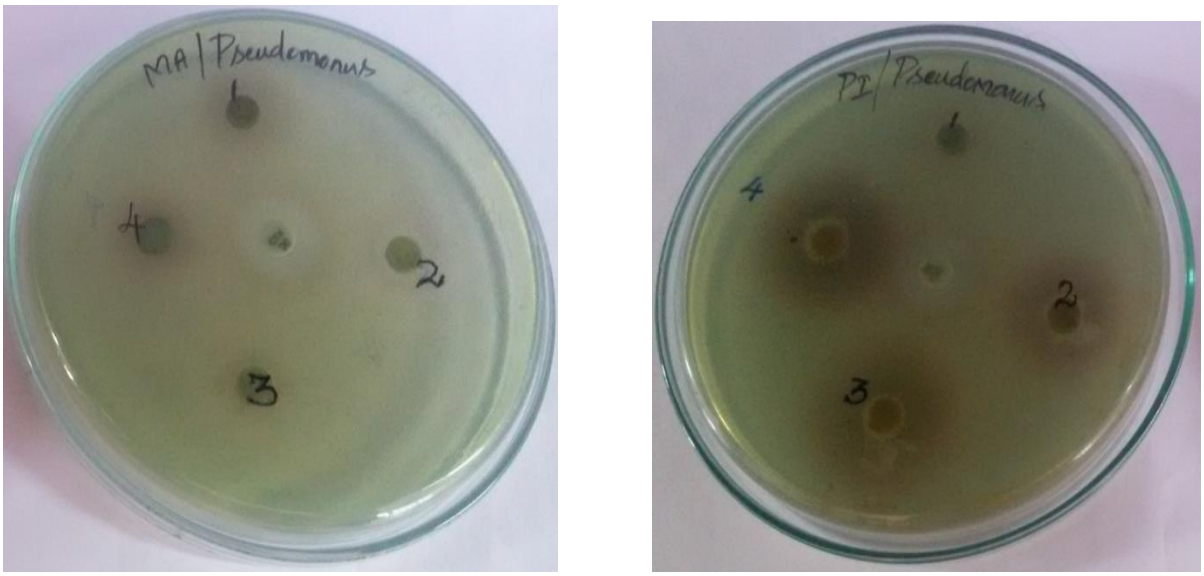


Figure No 9 Disc diffusion plate showing zone of inhibition of HAEMA & HAEPI against *Pseudomonas sp.*

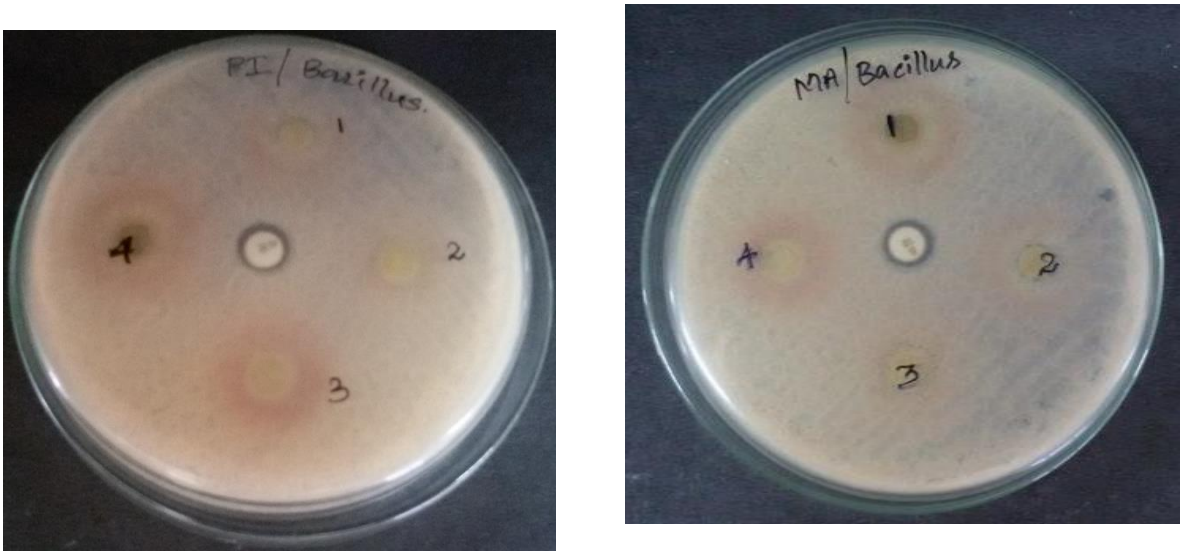


Figure No 10 Disc diffusion plate showing zone of inhibition of HAEMA & HAEPI against *B. subtilis*.

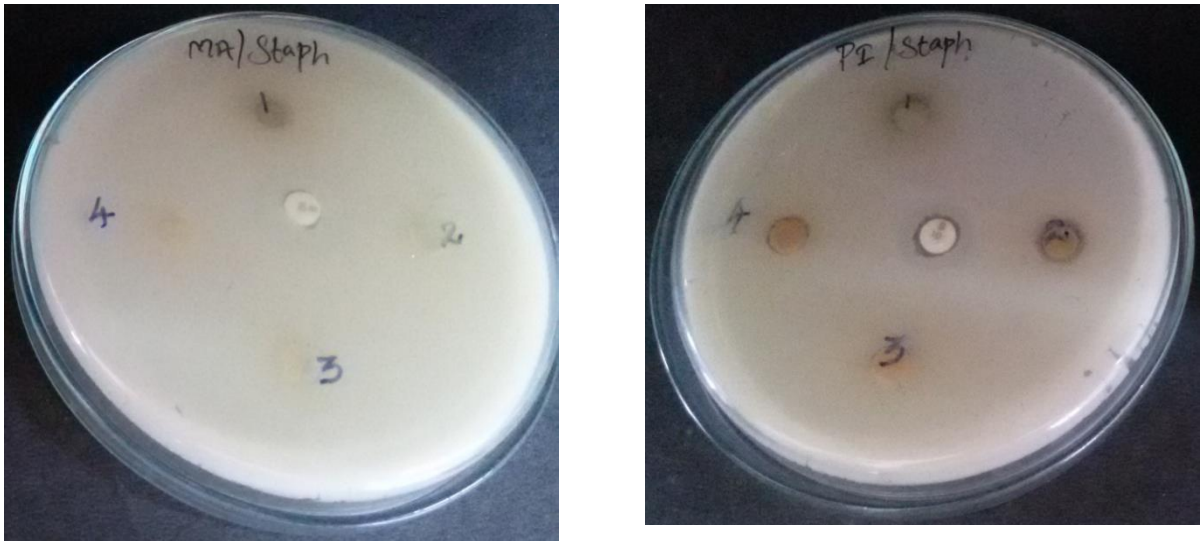


Figure No 11 Disc diffusion plate showing zone of inhibition of HAEMA & HAEPI against *S. aureus*.

7.3 Antibacterial Minimum inhibitory concentration (MIC) of hydroalcoholic extracts of *Martynia annua* L. and *Pentanema indicum*

Both the extracts of *Martynia annuna* L. and *Pentanima indicum* were tested against the following species, *E.coli, P.aruginosa, B.subtilis* and *S.aureus*. The HAEMA was found the Minimum Inhibitory Concentration in P.aruginosa (Gram-ve) at 7.81mg/ml.

The HAEPI was found minimum inhibitory concentration in *E.coli* (Gram –ve) at 62.5mg/ml. In this study reveals that the both plant extracts were moderate effective against *E. col.i* (Gram –ve species). (Table 3 & Figure 12,13)

Figure No. 12 Minimum inhibitory concentration of HAEMA

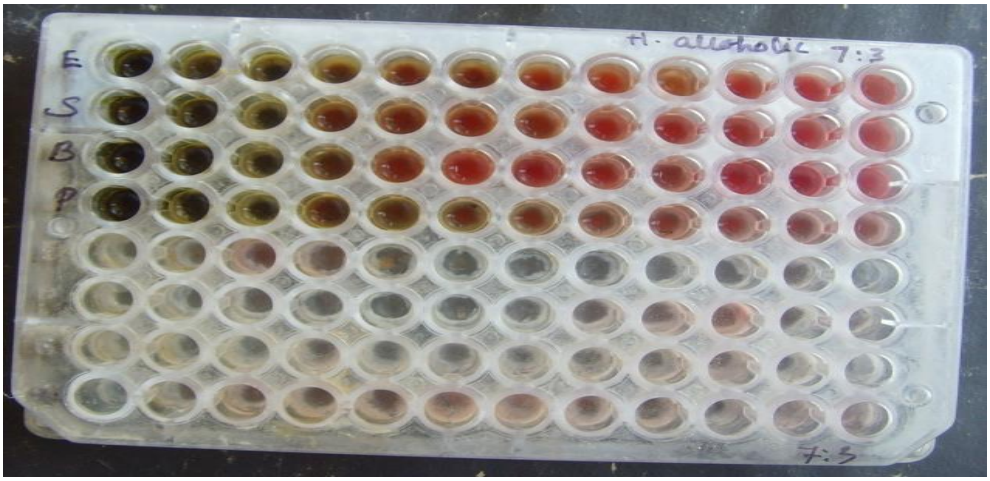


Figure No. 13 Minimum inhibitory concentration of HAEPI

Table 5 : Antibacterial Minimum inhibitory concentration (MIC) of hydroalcoholic extracts of *Martynia annua* L. and *Pentanema indicum*

Bacterial Species	MIC (mg/ml)	
	HAEMA	HAEPI
E.coli	15.62	62.5
Pseudomonas sp.	7.81	125
Bacillus subtilis	125	125
S.aureus	32.5	125

7.4 Antibacterial synergistic action of hydroalcoholic extracts of *Martynia annua* L. with *Pentanema indicum*

As compared to the individual extracts zone of inhibition the combination of HAEMA & HAEPI (1:1) posse significant increase in zone of inhibition in all four tested bacterial species. (Table 4 & Figure 14, 15, 16, 17)

Table 6 : Antibacterial synergistic action of hydroalcoholic extracts of *Martynia annua* L. with *Pentanema indicum*

Bacterial Species	Zone of inhibition in mm		
	HAEMA	HAEPI	HAEMA + HAEPI (1:1)
E.coli	7	6.2	9.6
Pseudomonas sp.	7.4	7.8	10.2
Bacillus subtilis	7.2	9	11.5
S.aureus	6	8.2	11

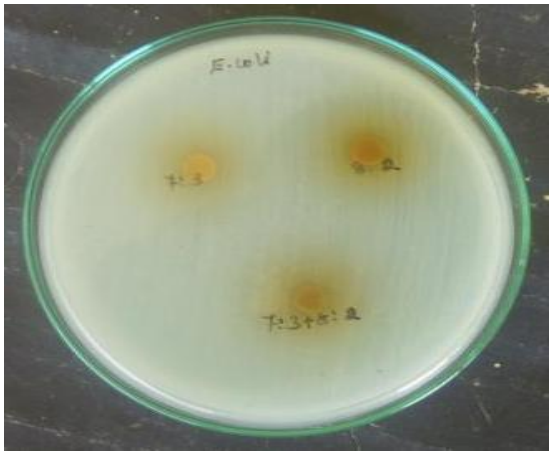


Figure No .14 Combination effect of plant extracts on *E.coli*

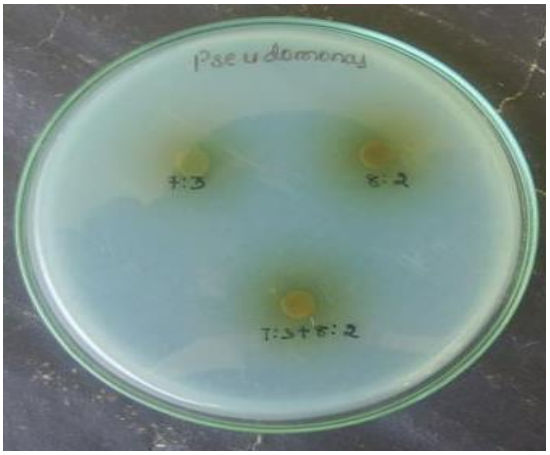


Figure No. 15 Combination effect of plant extracts on *Pseudomonas sp*.

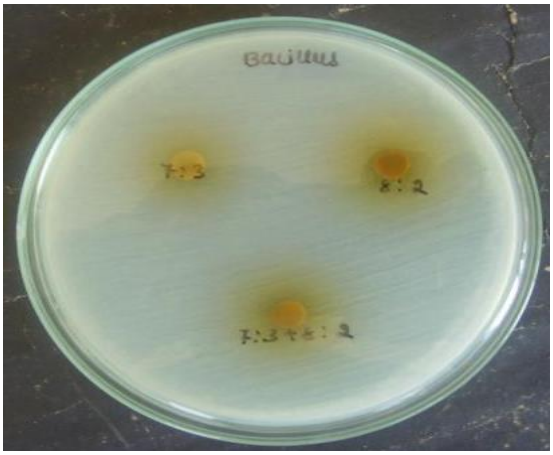


Figure No.16 Combination effect of plant extracts on *B.subtilis*

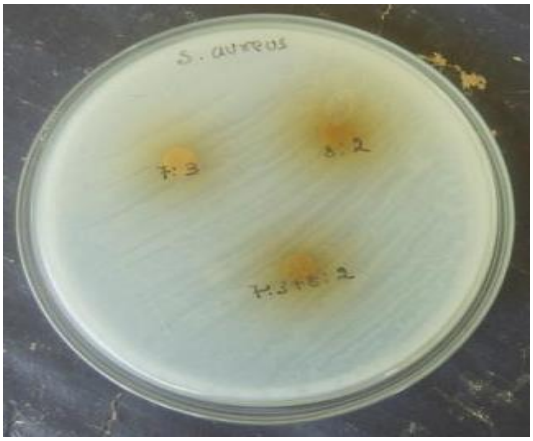


Figure No .17 Combination effect of plant extracts on *on S.aureus*

7.5 Antifungal activity of hydroalcoholic extracts of *Martynia annua* L. and *Pentanema indicum*

In this study the fungal species of *Candida* albicans and *Candida kruzei* were selected and Itaconazole (10 mg/ml) was used as standard.

HAEMA and HAEPI didn't show significant zone of inhibition on *Candida albicans* and *Candida kruzei* as compared to the standard Itraconazole (10 µg/ml). (Table 5 & Figure 18, 19, 20, 21)

Table 7 : Antifungal activity of hydroalcoholic extracts of *Martynia annua* L. and *Pentanema indicum*

Fungus Species	Zone of inhibition in mm								
	HAEMA (mg/ml)				HAEPI (mg/ml)				Standard
	50	100	150	200	50	100	150	200	Itraconazole (10 µg/ml)
Candida albicans	5	5.8	6.1	6.4	5	5.4	6.2	6.5	9.4
Candida kruzi	5	5.2	5.8	6.2	5	5.2	6.5	6.7	10.5

7.6 Antifungal Minimum inhibitory concentration (MIC) of hydroalcoholic extracts of *Martynia annua* L. and *Pentanema indicum*

Hydro alcoholic extracts of *Martynia annua L. and Pentanema indicum* were possesses the minimum inhibitory concentration at 250mg/ml against *Candida* albicans and *Candida kruzei*. (Table)

Table 8 : Antifungal Minimum inhibitory concentration (MIC) of hydroalcoholic extracts of *Martynia annua* L. and *Pentanema indicum*

Fungus Species	MIC (mg/ml)	
	HAEMA	HAEPI
Candida albicans	250	250
Candida kruzi	250	250

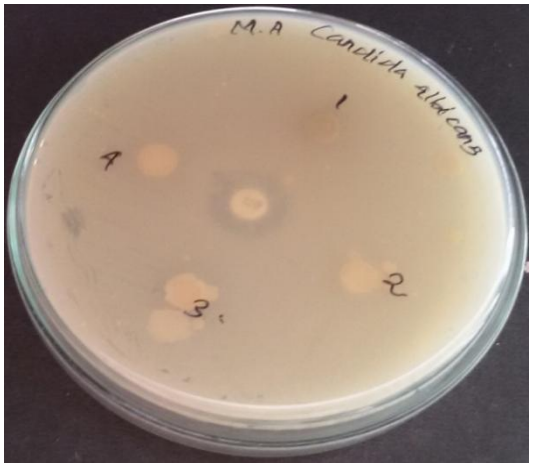


Figure No .18 Disc diffusion plate showing zone of inhibition of HAEMA against *candida albicans*

Figure No. 19 Disc diffusion plate showing zone of inhibition *of HAEPI against candida albicans*

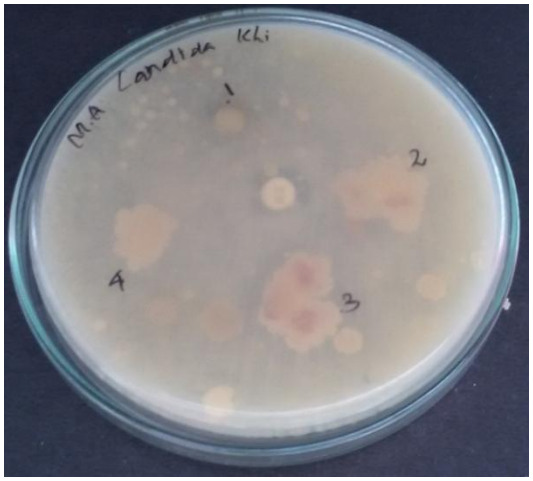


Figure No. 20 *Disc diffusion plate showing* zone of inhibition *of HAEMA against candida kruzei*

Figure No. 21 Disc diffusion plate showing* zone of inhibition *of HAEPI against candida kruzei

7.7 Antifungal synergistic action of hydroalcoholic extracts of *Martynia annua* L. with *Pentanema indicum*

The synergistic action of hydroalcoholic plant extracts were subjected to antifungal activity. The combination of HAEMA with HAEPI extracts (1:1) concentration to exhibiting the milder antifungal activity than the individual plant extracts. (Table 7)

Table 9 : Antifungal synergistic action of hydroalcoholic extracts of *Martynia annua* L. with *Pentanema indicum*

Fungus Species	Zone of inhibition in mm		
	HAEMA	HAEPI	HAEMA + HAEPI (1:1)
Candida albicans	6	7	10
Candida kruzi	8	7	12

7.8 *In-Vitro* Anthelmintic activity of hydroalcoholic extracts of *Martynia annua* L. and *Pentanema indicum*

The hydroalcoholic extract of *Martynia annua L. and Pentanema indicum* plants were subjected to the anthelmintic activity and compared with the standard drug Albendazole (Table 8 & Figure 22).

The extracts possess significant anthelmintic activity at the concentration of 10, 20, and 50 mg/ml in a dose dependent manner. In the HAEMA 10, 20, 50 mg/ml shows mild anthelmintic activity longer time for paralysis and death time. If they demonstrated shorter time of paralysis and death time at the concentration of 50mg/ml of hydroalcoholic extract caused paralysis of HAEPI at 7.33 ± 1.45 min and death at 14.00 ± 2.31 min. While albendazole as a positive control caused time of paralysis 20.67± 1.76 min and death time 32.0 ± 3.21min.And the combination plant extract caused time paralysis 6.00 ± 1.15 min and death 12.00 ± 1.15min well effective anthelmintic activity when compare the positive control. From the study, it was also clear that the time for paralysis and death decreases as the HAEPI at the 20,50mg/ml concentration of the extract (Table 8). Therefore the results demonstrate that the combination plant extract to posses the effective anthelmintic activity and thus may be used as an anthelmintic.

Table 10 : *In-Vitro* Anthelmintic activity of hydroalcoholic extracts of *Martynia annua* L. and *Pentanema indicum*

Treatment Group	Concentration (mg/ml)	Time taken for paralysis (in min)	Time taken for death (in min)
Control (normal saline)	-	-	-
Albendazole	20	20.67 ± 1.76	32.00 ± 3.21
HAEMA	10	53.33 ± 4.41	107.33 ± 3.71
	20	28.00 ± 6.25	70.00 ± 2.89
	50	25.00 ± 2.89	41.00 ± 2.08
HAEPI	10	15.00 ± 1.15	34.00 ± 2.89
	20	11.33 ± 1.45*	20.00 ± 3.46**
	50	7.33 ± 1.45**	14.00 ± 2.31***
HAEMA + HAEPI	50 + 50 (1:1)	6.00 ± 1.15**	12.00 ± 1.15***

Values are expressed as Mean ± SEM, n=3, Values were found out One way ANOVA followed by paried t-test. Symbol represents statistical significance:
* P<0.05 ** P<0.01 *** P<0.001

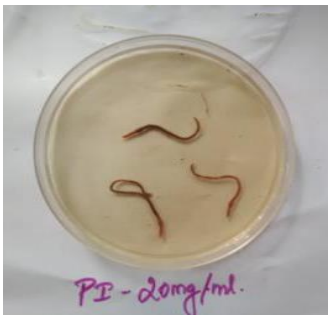
Albendazole (20mg/ml)

HAEMA (10 mg/ml)

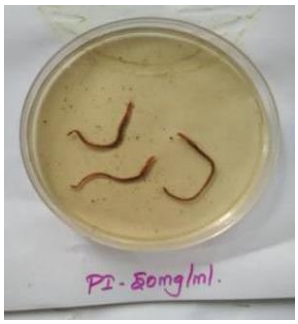
HAEMA (20 mg/ml)

HAEMA (50mg/ml)

HAEPI (10 mg/ml)



HAEPI(20 mg/ml)



HAEPI (50 mg/ml)

HAEMA + HAEPI(50 + 50 mg/ml)

Figure No. 22 Anthelmintic activity of hydroalcoholic extracts of *Martynia annua* L. and *Pentanema indicum*

7.9 *In-Vitro* Immunomodulatory activity (Phagocytosis stimulation) of hydroalcoholic extracts of *Martynia annua* L. and *Pentanema indicum*

In the study of immunomodulatory activity of test plant extracts evaluated for the ability to enhance phagocytosis. They showed efficacy in enhancing the phagocytosis function of human neutrophils to engulf *Martynia annua L*. with the phagocytosis stimulation of 83% by engulfing at 10 mg/ml concentration. The increase in number of engulfment of *Candida albicans* shows the phagocytosis will enhance the potency of the extracts. This was followed by the hydro alcoholic extract of *Pentanema indicum*, which showed 92%. And then the combination of the both plant extracts showed stronger efficacy in phagocytosis stimulation of 94% by engulfing at (10+10 mg/ml) concentrations.

Therefore the result was to determine that combination plant extract to posses stimulation of phagocytosis was increases then immunostimulating activity of *Candida albicans* to evaluated. (Table 9 & Figure 23).

Table 11 : *In-Vitro* Immunomodulatory activity (Phagocytosis stimulation) of hydroalcoholic extracts of *Martynia annua* L. and *Pentanema indicum*

Treatment Groups	Phagocytosis stimulation % (5mg/ml)	Phagocytosis stimulation % (10mg/ml)
Control (PBS)	-	-
HAEMA	83.3 ± 2.34	91.3 ± 4.57
HAEPI	82.4 ± 3.42	92.3 ± 6.54
HAEMA + HAEPI (10 + 10 mg/ml)	88.0 ± 6.41	94.4 ± 1.42

Values are expressed as Mean ± SEM, n=3.

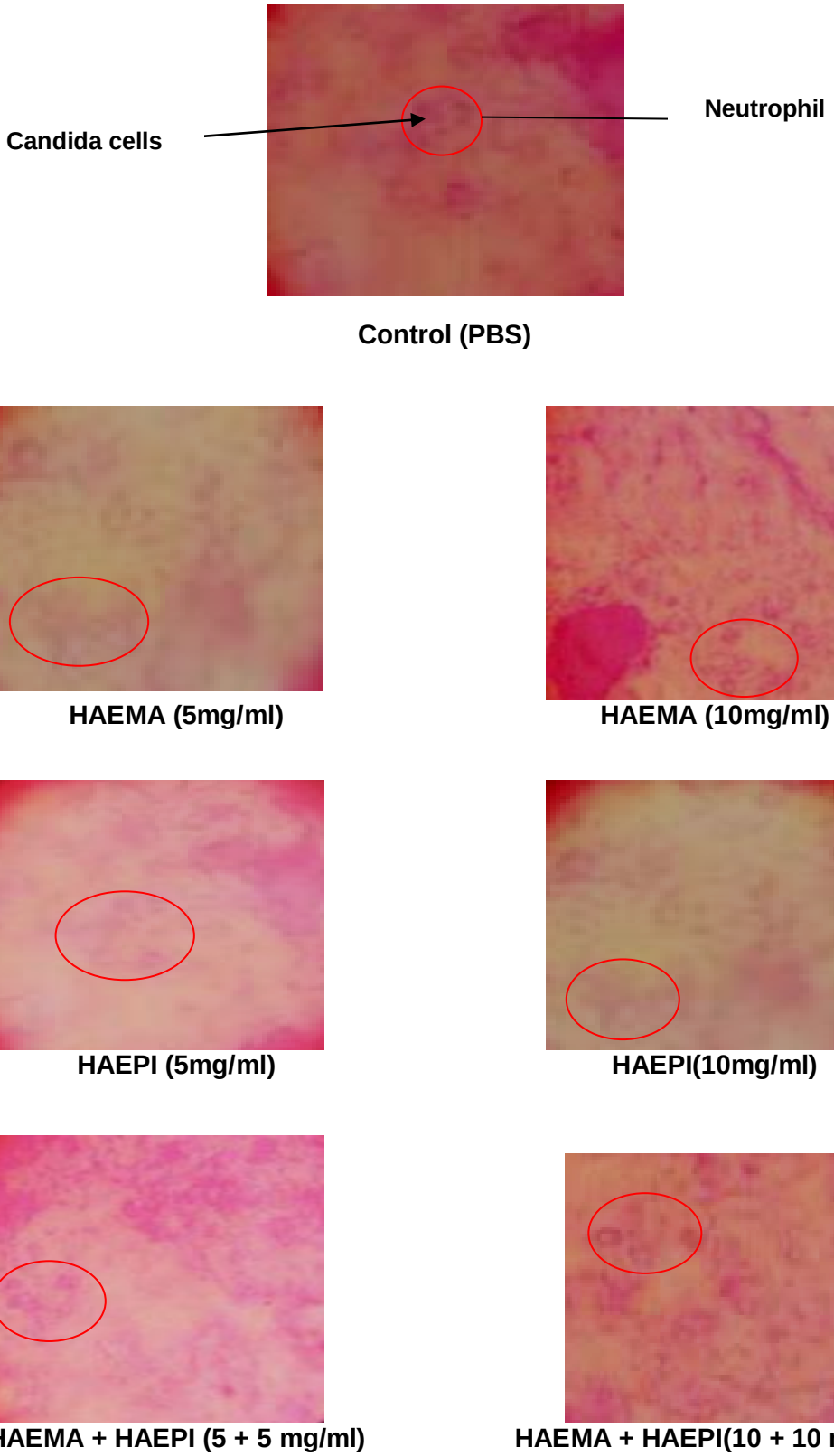


Figure No. 23 Immunomodulatory activities (Phagocytosis stimulation) of hydroalcoholic extracts of *Martynia annua* L. and *Pentanema indicum*

CHAPTER-8

DISCUSSION

CHAPTER – 8

DISCUSSION

The therapeutic potential and other pharmacological actions of phytomedicines are due to their therapeutically active constituents such tannins, alkaloids and several other aromatic compounds or the secondary metabolites of plants. So the preliminary phytochemical analysis revealed pronounced importance to explore the composition of secondary metabolites and active constituents in crude drugs. In the present study, preliminary phytochemical screening of hydroalcoholic extracts of *Martynia annua* L. and *Pentanema indicum* showed significant indication about the presence of metabolites like alkaloids, saponins, tannins, amino acids, flavonoids, terpenoids, carbohydrates, glycosides and gum mucilage. Most of the previous study reported that plants with flavonoids and alkaloids are commonly found to be having antimicrobial properties[111].The result of the present study also supplements the folkloric usage of these studied plants which possess several known and unknown bioactive compounds with bioactivity. So the antimicrobial activity of the HAEMA and HAEPI as recorded in present study might therefore be attributed to the presence of flavonoids, alkaloids, terpenoids and carbohydrates.

In this study, antibacterial activity of hydroalcoholic extracts of *Martynia annua* L. and *Pentanema indicum* were evaluated at the concentrations ranges are 50, 100, 150, 200 mg/ml. The extracts were screened against Gram – positive (*Bacillus subtilis, Staphylococcus aureus*), Gram – negative (*Escherichia coli, Pseudomonas aeruginosa*) bacteria and results were compared with the standard drugs such as Bacitracin 10µl/ml (gram negative), cefotaxime 10 µl/ml (gram positive) by disc diffusion method. The zone of inhibition was seen in hydroalcoholic extracts against all cultures in order of *Martynia annua* L. shows P.aeruginosa > *E.coli* > *S.aureus* > *B.subtilis*, HAEMA shown the maximum inhibition against the gram negative bacteria *P.aeruginosa*. *Pentanema indicum* shows the zone of inhibition in the order of *B.subtilis* > *S.aureus* > P.aeruginosa > *E.coli,* HAPI shows the maximum inhibition against the gram positive bacteria *E.coli*. The reason for the difference sensitivity between the gram positive and gram negative bacteria could be ascribed to the morphological differences between these microorganisms, gram negative pathogens

having an outer 600 Da. The gram positive bacteria should be more susceptible having only an outer peptidoglycone layer, which is not an effective permeability barrier[112]. So HAEPI possess the more sensitivity against gram positive organisms better than HAEMA and HAEPI exhibited the antibacterial activity against gram negative organisms lesser than HAEMA. This result based on the phytoconstituents present in the HAEMA & HAEPI mainly alkaloids and flavonoids compounds are possess the antibacterial activity.

Minimum inhibitory concentration (MIC) of the crude hydroalcoholic extracts was determined by checkerboard assay method. This method also gives the opportunity to determine an approximate MIC indicating the degree of potential antimicrobial activity. The results showed in the order of test bacteria cultures *E.coli, P.aerugenosa, S.aureus, B.subtilis*. The activity of HAEMA against the *E.coli* MIC value 15.62 mg/ml, *P.aeruginosa* MIC value 7.81 mg/ml, *B.subtilis* value 125 mg/ml, *S.aureusi* MIC value 32.5 mg/ml showed. Less concentration of HAEMA possess the inhibition of *P.aeruginosa* (7.81 mg/ml), so HAEMA possess higher sensitivity to the *P.aeruginosa* as compared with other test bacterial culture. The activity of HAEPI against the *E.coli* MIC value 62.5 mg/ml, *P.aeruginosa* MIC value 125 mg/ml, *B.subtilis* value 125 mg/ml, *S.aureusi* MIC value 125 mg/ml showed. Results are HAEPI sensitive to the *E.coli* (62.5 mg/ml), HAEPI possess the maximum inhibition of *E.coli* comparisons with another bacterial cultures[113].

Combination (1:1) of HAEMA and HAEPI shows synergistic activity against *E.coli, P.aerugenosa, B.subtilis and S.aereus.* Combination treatment group shows better activity against all cultures as compared with individually treated groups. Combination of extracts exerts maximum zone of inhibition against Gram +ve and G-ve organisms as compared to individual extract activity. So this combination exerts broad spectrum antibacterial activity. Synergistic activity is revealed that better than the individually treatment groups concluded by based on the exhibited activity report[114].

Hydroalcoholic extract of *Martynia annua* L. and *Pentanema indicum* at concentrations range (50, 100, 150, 200 mg/ml) against the *Candida albicans* and *Candida kruzei* fungal culture compared with standard drug by disc diffusion method.

All concentration of HAEMA and HAEPI against *Candida albicans* and *Candida kruzei* does not exhibit prominent zone of inhibition as compared to the standard. But the combination of HAEMA and HAEPI (1:1) ratio possess the milder antifungal activity, compared with individually treated group of plant extract.

Helminthiasis, the condition resulting from worm infestation is one of the major prevalent diseases in the world, particularly in the tropical countries. The best alternative over modern synthetic drugs is plant derived medicine. Many investigators submitted their work on the similar aspect and their reports support this investigation revealing that plants are potent anthelmintic agents[116]. It was observed from the study that, the hydroalcoholic extracts of *Martynia annua* L and *Pentanema indicum* plant demonstrated anthelmintic activity. Concentration of extracts (10, 20, 50 mg/ml) and standard drug albentazole (20 mg/ml) were used in this study. The test samples of HAEMA and HAEPI all concentration exhibited significant dose dependent anthelmintic activity in earthworms in comparison to that of the standard of albentazole. Best action obtained in 50 mg/ml of HAEPI (14 ± 2.31min) The findings of this test results revealed that, the extract exhibited not only paralysis but also death of earthworms and the calculated time for paralysis and death of earthworms were inversely proportional to the plant extract concentration. Combination of HAEMA and HAEPI (1:1) 50+50 mg/ml possess the significant activity compared with standard drug. Previous study data reported that, the presence of phenols, tannins, alkaloids, terpenoids may be responsible for exhibit anthelmintic activity[117]. Therefore, albentazole is cellular integrity tubulin inhibitor type of broad spectrum anthelmintic activity. Likely, test sample possess this type of inhibition were performed, mechanism was not properly further study requires to define the mechanism of these plant extracts. The HAEPI exhibited the more significant activity than standard and MA alone treated group. In comparisons Combination and HAEPI possess more significant anthelmintic as comparison with others.

The test plant extracts evaluated for their ability to enhance phagocytosis showed that HAEMA and HAEPI to be potent. Concentration of HAEMA and HAEPI plant extract at the concentration of (5, 10 mg/ml) showed stronger efficacy, by enhancing the phagocytosis function of human neutrophils to engulf *Candida albicans* with the phagocytosis stimulation percentage of HAEMA is 91.3±4.57 at 10

mg/ml, 83.3±2.34 at 5 mg/ml and HAEPI percentage of 92.3±6.54 at 10 mg/ml, 82.4±3.42 at 5 mg/ml concentarations. Test samples 5 mg/ml less potent than 10 mg/ml concentration were active with different phagocytosis stimulation ability. While remaining test extracts sidelined to provide any stimulation of human neutrophils to engulf more *Candida albicans* and showed the not effect as that of control with engulfing cells at determined concentrations. The present work of phagocytosis stimulation was similar with the[118], thus supports the present study. Phagocytosis enhancing potency of the HAEMA and HAEPI varies and may depend on the ability of the bioactive components in the extracts. Flavonoids, terpenoids[119], steroids and tannins[120] are some of the plant secondary metabolites found to have immunomodulatory activity and the obtained results may attribute to any of these bioactive compounds present in test extracts. The process of engulfment may depend on the receptor activation or chemotactic ability of these secondary metabolites present. The exact process is not yet properly understood, as a very less work has been done in this aspect. The final step of phagocytosis is the intracellular killing of microorganisms by the phagocytic cell[121]. The combination of HAEMA and HAEPI also exhibit the 94.4±1.42 at 10 mg/ml and 88.0±6.41 at 5 mg/ml engulfed of *Candida albicans* enhance the phagocytosis.

CHAPTER-9

SUMMARY AND CONCLUSION

CHAPTER - 9

SUMMARY AND CONCLUSION

Synthetic drugs are usually associated with various side effects. More attention is attracted by the increasing problems of development of resistance in infectious disease against synthetic drugs. The best alternative over modern synthetic drugs is plant derived medicine. In this study the two different hydroalcoholic extracts were subjected to the antimicrobial, antifungal, anthelmintic and immunomodulatory activities.

The plants were screened for phytochemical constituents, seemed to have the potential to act as a useful drugs and also to improve the health status of the consumers due to the presence of various compounds that are vital for good health. Based on the presence of phytochemical such as alkaloids, saponins, tannins, amino acids, flavonoids, terpenoids, carbohydrates, glycosides and gum mucilage the hydroalcoholic extracts of *Martynia annua* L. and *Pentanema indicum* were subjected to the various activities like antifungul, antimicrobial, anthelmintic and immunomodulatory was studied.

The hydroalcoholic extracts were analyzed for the antibacterial activity of plant extract against the microorganism (*E.coli, P.auregenosa, B.subtilis, S.aureus)* and compared with standard drug by disc diffusion method. Plant extracts possess the antibacterial activity in dose dependent manner at 50, 100, 150, 200mg/ml concentrations.

MIC by checker board assay is probably the most convenient way of assessing the antibacterial potential of plant extracts. Tested microorganisms (*E.coli, P.auregenosa, B.subtilis, S.aureus)* in the MIC value of HAEMA against *P.auregenosa* was found to be 7.81mg/ml, against *E.coli* its 15.62 mg/ml and on *S.aureus* its 32.5mg/ml. MIC value of HAEPI on *E.coli* found to be 62.5mg/ml it better than compared with other microorganism in same extract.

Combination of HAEMA and HAEPI (1:1) shows greater zone of inhibition against Gram +ve and Gram -ve organisms as compared to the individual extract effects, as HAEMA shows greater sensitivity against Gram -ve organism; HAEPI shows greater sensitivity against Gram +ve organism. Which revealed that combination possess broad spectrum in action as compared to the individual

extracts. It might be due to synergistic action of Hydroalcoholic extract of *Martynia annua* L. and *Pentanema indicum*.

Hydroalcoholic extract of *Martynia annua* L. and *Pentanema indicum* against *Candida albicans* and *Candida kruzei* fungal organism does not possess prominent antifungal action. But in combination it possess milder antifungal action, it might be due to synergistic action.

Hydroalcoholic extract of *Pentanema indicum* its shows more significant anthelmintic action against earth worm as compared to the HAEMA and broad spectrum albendazole standard. Also 1:1 combination of HAEMA with HAEPI also shows more significant anthelmintic action.

Immunomodulatory activity (phagocytosis stimulation) of hydroalcoholic extracts of *Martynia annua* L. and *Pentanema indicum* showed dose dependent increase in phagocytosis stimulation of human neutrophils against *Candida albicans*. Combination of plant extracts (10+10mg/ml) shows more significant increase in phagocytosis stimulation percentage (94.4%) as compared to the others. It might be concluded that both the extracts posses the phagocytosis stimulation action and more phagocytosis stimulation was observed over combination.

CONCLUSION

In conclusion hydroalcoholic extracts of *Martynia annua* L. and *Pentanema indicum* shows narrow spectrum against bacterial species and insignificant antifungal action but the combination possess broad spectrum of antibacterial and milder antifungal action. In cause helminthes infection *Pentanema indicum* alone or combination shows more significant anthelmintic action over broad spectrum albendazole. Combination of *Martynia annua* L. and *Pentanema indicum* extracts also posses more significant Immunomodulation (phagocytosis stimulation) action. So in case of unspecified microbial infections this multi potent plant extracts combination is much beneficial and nontoxic as compared to the synthetic medicines. Further isolation and characterization of phytoconstituents and *In -vivo* evaluations are need to explore this extracts as phytomedicines against pathogenic microorganisms.

CHAPTER-10

REFERENCE

CHAPTER - 10

REFERENCE

1. Seyyed Mansour Seyydnejad, Masumeh Niknejad, Ismaieel Darabpoor and Hossein Motamedi. Antibacterial activity of Hydroalcoholic Extract of *Calistemon citrinus* and *Albizia lebbeck*. *Am J of App Sci.* 2010; 7(1): 13-16.
2. Karthika Krishna Moorthy, Paulsamy Subramaniam and Jamuna Senguttuvan *Invitro* Antifungal Activity of Various Extracts of Leaf and Stem Parts of *Solena Amplexicaulis*(lam.) Gandhi. *Inter J of Pharmacy & Phama sci.* 2013; 5 (3): 34-39.
3. Said O, Khalil K, Fluder S, Azaizeh. Ethnobotanical survey of medicinal herbs of the Middle East region. *J Ethanopharamacol.* 2002; 83:251-6.
4. Shankar K, Liao LP. Traditional systems of medicine. Physical medicinal and rehabilitation clinics of North America. 2004; 15(4): 725-747.
5. Thillaivanan S, Samraj K. Challenges, Constraints and opportunities in herbal medicines – A review. *Inter J of Herbal Medicine*. www.florajournal.com. 2014; 2(1): 21-24.
6. Kamboj V P., Herbal Med Chinese Medicine*. Current Science* 2000; 78: 35-38.
7. Efferth T, Kaina B., Toxicities by herbal medicines with emphasis to traditional chinese medicine. *Curr Drug Metab*. 2011; 12(10):989-96.
8. Peters D. CAM: doing more good than harm. *Focus Altern Complement Ther.* 2009; 14: 176-8.
9. Usha PTA, Jose S, and Nisha AR. Antimicrobial drug resistance- a global concern. Veterinary World, 2010; 3: 138- 139.
10. Santram L, Abhay K S., Wound healing effect of flavonoids rich fraction isolated from *Martynia annua* Linn. *APJTM*.2013; 253-259.
11. Elakkia SA, Venkatesalu V. Antimicrobial activity of different solvent extracts of some *Cassia* species. Int J Pharma Bio Sci 2013; 4: 728‑36.
12. Sibaram P, Subhasish M, Mookerjee M. Phytochemial investigation and evaluation of anthelmintic activities of *V. Negundo* leaf extract. Int J Res Pharm Biomed Sci 2012; 3:1143‑6.
13. Mali RG, Mehta AA. 2008. A review on anthelmintic plants. Nat Prod Radi, 7: 466-475.

14. Iqbal Z, Nadeem QK, Khan MN, Akhtar MS, Waraich FN. 2001. *In vitro* anthelmintic activity of *Allium sativum, Zingiberofficinale, Cucurbitamexicana, Ficusreligiosa*. Int J Agri Biol, 3: 454-457.
15. Jackson F, Coop RL. 2000. The development of anthelmintic resistance in sheep nematodes. Parasitol, 120: S95-S107.
16. Eguale T, Tadesse D, Giday M. 2011. In vitro anthelmintic activity of crude extracts of five medicinal plants against egg-hatching and larval development of *Haemonchus contortus*. J Ethnopharmacol, 137: 108–113.
17. Rameshroo K, Pushpa P, Trilochan S, Amit R. *Martynia annua* : An Overview. 2013. 1-7.
18. Britto, John De A, Sebastain, Steena Roshan, Sujin Mary R*.,* Antibacterial activity of selected of lamiaceae against human pathogens. *Indian J of Nat Products and resources.* 2012; Vol 3(3): 334-342.
19. www.google.com. Medscribe
20. Apoorva Pahadia, Rakhi Gawde, Shikha Agrawal*.,* Antimicrobial Activity of Hydroalcoholic Extract of Areca Catechu.*Int J of Pharma Erudition*. 2013; 3(1):18-25.
21. Sohail, Zafar Iqbal, Muhammad Afzal, Aftab Afzal, Inayat Ur Rahman, Salma
22. Shad, Bilal Ahmed, Naveed Anjum, Kalsoom Qureshi, Afsana Bibi., *In vitro* antibacterial study of *Taraxacum officinale* leaves extracts against different bacterial pathogenic strains. *J of Pharmacognosy and Phytochemistry*. 2014; 3(2): 15-17.
23. Neethu SK, Neethu Simon., *In vitro* antibacterial activity and phytochemical analysis of *Gliricidia sepium* (L.) leaf extracts. *J of Pharmacognosy and Phytochemistry*. 2016; 5(2): 131-133.
24. Mahesh B, Satish S., Antimicrobial Activity of Some Important Medicinal Plant Against Plant and Human Pathogens. *W J of Agri Sciences.* 2008; 4(S): 839-843.
25. Tariro AC, Stenley M., *In vitro* Antibacterial Activity of Selected Medicinal Plants from Zimbabwe. *The Af J of Plant Science and Biotechnology.* 2011; 5(1): 1-7.
26. Gutal Valyfathulla Shaik., Antifungal Activity of An Indian Medicinal Plant Argyreia Involucrata. *Int J of Res in Pharma and Bio Sci*. 2011; vol 2(4): ISSN: 2229-3701.

27. www.google.com. search
28. Hasan M F, Das R, Alam KH, Hossain MS and Rahman M.The Determination of Antibacterial and Antifungal Activities of *Polygonum hydropiper*(L.) Root Extract. *Advances in Biological Research*. 2009; 3(1-2): 53-56.
29. Duraipandiyan V, Ignacimuthu S., Antifungal activity of traditional medicinal plants from Tamil nadu. *Asian Pacific J of Tropical Biomedicine.* 2011; S204-215.
30. Gutal VS., Antifugal Activity of An Indian Medicinal Plant *Argyreia Involucrata*. *Int J of R in Pharma and Biomedical Sciences.* 2011; Vol 2(4):ISSN: 2229-3701.
31. Harison Masih, Jyotsna Kiran Peter and Pratima Tripathi*.,* A Comparative evaluation of antifungal activity of medicinal plant extracts and chemical fungicides against four plant pathogens. *Int J Curr Microbiol App Sci*.2014;3(5): 97-109.
32. Idika JK, Okonkwo EA, Onah DN, Ezeh IO, Iheagwam CN, Nwosu CO*.,* Efficacy of Levamisole and Ivermectin in the control of bovine parasitic gastroenteritis in the sub-humid savanna zone of southeastern Nigeria. *Parasitol Res. 2012; 111(4):1683-7.*
33. Lukhoda CW, Simmonds MS, Paton AJ*.,* Plectranthus: A review of ethnobotanical uses. *J Ethnopharmacol*. 2006; 103(1): 1-24.
34. Keiser J and Utzinger J*.,* Efficacy of current drugs against soil-transmitted helminthes infections: systematic review and meta-analysis. *JAMA.* 2008; 299(16): 1937-1948.
35. Charlier J, Van Der Voort M, Kenyon F, Skuce P, Vercruysse J., Chasing Helminthes and Their economic impact on farmed ruminants. *Trends Parasitol.* 2014; 30(7):361-367.
36. Fitzpatrick JL., Global food security: the impact of veterinary parasites and parasitologists. *Vet parasitol*. 2013; 195(3-4):233-248.
37. Sutherland IA, Leathwick DM. Anthelmintic resistance in nematode parasites of cattle: a global issue. *Trends parasitol.2011;* 27(4):176-181.
38. Sarison ND. Pharamceutical treatments of gastrointestinal nematode infections of sheep- future of anthelmintic drugs. *Ver Parasitol.* 2012; 189(1):79-84.

39. Githiori JB, H glund J, Waller PJ. Ethnoveterinary plant preparations as livestock dewormers: practices, popular beliefs, pitfalls and prospects for the future. *Anim Health Res Rev*. 2005; 6(1): 91-103.
40. Athanasiadou S, Githiori J, Kyriazzakis I. Medical plants for helminth parasite control: facts and ficion. *Animal*. 2007; 1(9):1392-1400.
41. Tollossa K, Debela E, Athanasiadou S, Tolera A, Ganga G, Houdijik J. Ethnomedical study of plants used for treatment of human and livestock ailments by traditional healers in south Omo. Southern Ethiopia. *J Ethnobiol Ethnomed.* 2013; *9(1):32.*
42. Cowan MM., Plant Products as Antimicrobial Agents. *Clin Microbiol Rev.* 1999; 12(4):564-582.
43. Muller- Harvey I., Unravelling the conundrum of tannins in animal nutrition and health. *J Sci Food Agri.* 2006; 86(13):2010-2037.
44. Martinez - Micaelo N, Gonazafez-Albuin N, Ardevol A, Pinent M, Blay MT., Procyanidins and inflammation: Molecular targets and health implications. *Biofactors*. 2012; 38(4): 257-265.
45. Gonzaler R, Ballester I, Lopez-posadas R, Suarez MD, Zarzuelo A, Martinez-Augustin O, Media FSD., Effects of flaconoids and other polyphenols on inflammation. *Crit Rev Food Nutr*. 2011; 51(4):331-362.
46. Butter NL, Dawson JM, Wakelin D, Buttery PJ., Effect of dietary condensed tannins on gastrointestinal nematodes. *J Agric Sci.* 2001; 137(04):461-469.
47. Heckendorn F, Haring DA, Maurer V, Zinsstag J, Langhnas W, Hertzberg H., Effect of sainfoin (*Onobrychis Viciifolia)* Silage and hay on established polpulations of *haemonchus contortus* and *cooperia curticei* in lambs. *Vet Parasitol.* 2006; 142(3-4): 293-300.
48. Brunet S*,* Jackson F, Hoste H., Effects of sainfoin (*Onobrychis viciifolia)* extract and monomers of condensed tannins on the association of abomasal nematode larvae with fundic explants. *Int J Parasitol.* 2008; *38(7): 783-790.*
49. Novobilsky A, Mueller – Harvey I, Thamsborg SM., Condensed tannins act against cattle nematodes. *Vet Parasitol.* 2011; (38(7):783-790.
50. Williams AR, Fryganas C, Ramsay A, Muller – Harvey I, Thamsborg SM. Direct anthelmintic effects of condensed tannins from diverse plant sources against *Ascaris suum. PLOS One.* 2014; 9(5): e97053-10.1371/jornal. Pone.0097053.

51. Kishor K, P. Satheesh Kumar and T.Venkatachalam. Investigation of Anthelmintic Activity of *Pergularia Daemia* Leaves. *Pharamacophore*. 2014; 5(1): 44-48.
52. Ashok K, Lakshman K, Jayaveera KN, Nandeesh R, Manoj, B. And Ranganayakulu D. Comparative *In Vitro* Anthelminitic Activity of Three Plants From The Amaranthaceae Family. *Arch Biol Sci*. 2010; 62(1):185-189.
53. Saju T, Dr. Seetha Devi B. Phytochemical and *In vitro* Anthelmintic Studies of Hydro-Alcoholic Extract of *Costus Pictus* D. Don. *Int J of Pharmacy and Pharmaceutical Sciences*. 2013;Vol 5: Issue 3.
54. Kalyani N, Sayali Mutalik, Shajmeen Attar, Minakshi Nehete and Pratima Tatke. *In vitro* Anthelmintic Activity of some Medicinal Plant Extracts. *EJPMR*. 2016; 3(11): 633-636.
55. Hotchkiss RS, Nicholson DW. *Nat Rev Immunol.* 2006; 6(11): 813-22.
56. Matute-Bello G, Frevert CW, Martin TR. *Am J Physiol Lung Cell Mol Physiol*. 2008; 295(3): L379-99.
57. Wan L, Bagshaw S M. Langenberg C*. Crit Care Med.* 2008; 36(4): S198-203.
58. Donald AM, adamis D, Treloar A. *Age Ageing*. 2007; 36: 222-25.
59. Annane D, Bellisant E, Cavaillon JM. *Lancet.* 2005; 365: 63-78.
60. Hotchkiss RS, Nicholson DW. *Nat Rev Immunol.* 2006; 6(11): 813-22.
61. Roitt I, Brostoff E, Male D. Immunology. *J.B. Lippincott Co;* 1989:2.
62. AMFAR, AIDS/HIV Treatment Directory. 1993; 6:4.
63. Mosmann TR, Coffman RL. *Annual Review of Immunology*. 1989; 7: 145-173.
64. Yarchoan R, Mitsuya H, Broder S. *Immunology Today.* 1993; 14:303-309.
65. Wood R. *J of Infection Dis.* 1993; 167: 519-525.
66. Francis ML, Meltzer MS, Gendlman HA. *AIDS Res Human Retroviruses.* 1992; 9: 199-207.
67. Schroder K, Hertzog PJ, Ravasi T, Hume DA. *J Leukoc Biol.* 2004; 75(2): 163-189.
68. Hill N, Sarvetnick N. *Curr Opin Immunol*. 2002; 14(6): 791-797.
69. Bach JF. *Transplant Pro*. 1996; 28(6): 3023-3025.
70. Szekeres-Bartho J, Barakonyi A, Par G, Polgar B, Palkovics T, Szereday. *Int Immunopharamacol.* 2001; 1(6): 1037-1048.
71. Abo-Zena RA, Horwitz ME. *Curr Opin Pharamcol.* 2002; 2(4): 452-457.

72. Roberts CW, Satoskar A, Alexander J. *Parasitol Today*. 1996; 12(10): 382-388.
73. Nizametidinova G. *Reports of the Kazan Veterinary Institute*. 1972; 112:100-104.
74. Graber JM, Goust AD, Glassman, Kendall R, Loadholt CB. *J Infect Dis.* 1981; 143(1):101-105.
75. Reap EA, Lawson JW. *J Lab Clin Me.* 1990; 115(4): 481-486.
76. Weiss RC. *Am J Vet Res.* 1992; 53(5): 829-833.
77. Arya V, Gupta VK. *Int J of Pharmacy & Life Sci.* 2011; 2(5): 751-758.
78. Agarwal S, Singh VK. Part:1 Medicinal plants. *PINSA*. 1999; B 65(3-4): 179-204.
79. Billiau A, Matthys P. *J of Leukocyte Biology.* 2001; 70: 850-860.
80. 79.Susan FM, Sally RS. Introductory Clinical Pharmacology 27 edition. *Lippincott Williams and Wilkins.USA.* 2009; 567-568.
81. Susan FM, Sally RS. Introductory Clinical Pharmacology 27 edition. *Lippincott Williams and Wilkins.USA.* 2009; 667-668.
82. Attard E and Cuschieri A. *In vitro* immunomodulatory activity of various extracts of Maltese plants from the Asteraceae family. *J of Med Plants Res.* 2009; Vol-457-461.
83. Sheikh Abid Ali, Aruna Bhatia, Qazi Parvaiz, H. M. Bhat, Sheikh Fayaz Ahmad, Nishu Khera and Refaz Ahmad. *In vitro* immunomodulatory study of different parts of *Prunus cerasus* L. (sour cherry) Plant. Asian J of Plant Science and Res. 2013; 3(3): *35-43.*
84. Vanita G Kanase, Brijendra B Jain, Pramila Yadav. Evaluation of *In vitro* Immunomodulatory Activity of Hydroalcoholic Extract of *Ceropegia Bulbosa Roxb. Int J of Res and Scientific Inno*. 2013; Vol III: Issue X.
85. Boudoukha C *et al.,* Immunomodulatory effects of santolina chamaecyparissus leaf extracts on human neutrophil fynctions. *Pharmaceu Bio.* 2015; 1-6
86. Bhanwase A S., Antioxidant and Immunomodulatory activity of hydroalcoholic extract and its fractions of leaves of Ficus benghalensis L. 2016; Vol 8: issue 1, 50-55.
87. Rameshroo K, Pushpa P, Trilochan Satapathy. *Martynia annua* : An Overview. *Martynia annua Phytoconstituents.* 2013; 1-7.
88. Ashwani KD *et al.,* Martynia annua: A Review. *J of Pharmacognosy and Phytochemistry.* 2013; Vol 1: P.No 135.

89. Suryawanshi, Tare HL., Phytochemical Screening and Antibacterial Activity of Martynia annua Unripe Fruits and Flower Extracts. *Int J of U Pharmacy and Life Sciences.* 2013; 3(2).
90. Kalaichelvi K, Dhivya SM., Phytochemical Screening and Antibacterial activity of leaf extract of Martynia Annua, L. and Premna latifolia, Roxb. *J of Med Plants Studies.* 2016; 4(4): 84-87.
91. Padmapriya M *et al.,* phytochemical Screening Study of Martynia Annua. *IJPPR.* 2016; 7(1): 23-28.
92. Aziz-ur-Rehman *et al.,* Martynia annua : Comparative Antioxidant Potential of Its Stem and Leaves. *As J of Chemistry.* 2012; vol 24:P.No:8.
93. www.indianetzone.com. Reference. Flora & fauna. Indian medicinal plants.
94. Khandelwal K R. Practical Pharmacognosy. Nirali Prakashan.1995; 149-155.
95. Evans W C, Trease G E. Trease and Evans Pharmacognosy. WB Sauders. China. 2002; 193-407.
96. Kokate C K, Purohith A P, Gokhale S B. Pharamacognosy. Nirali Prakashan 2009; 6: 6-10.
97. De S, Dey YN, Ghosh AK. Phytochemical investigation & Chromatographic evaluation of different of extract of tuber of *amorphophallu paeoniifolius* (Araceae). *Int J Pharm & Biomedical Res.* 2010; 1(5): 150-157.
98. Sahu Vinod K, Irrchaiya Raghuveer, Shashi Alok, Gurjar Himanshu. Phytochemical investigation and chromatographic evaluation of thr ethanolic extract of whole plant extract of *Dendrophthoe falcate* (L.F) Ettingsh. *Int J of Pharma Sci and Res*. 2010; 1(1): 39-45.
99. Harborne JB. (Eds). Phytochemical methods: A guide to Modern techniques of Plant analysis. 3rd ed. Chapman and hall, London. ISBN : 0-412-57270-2. 1998; 302.
100. Shanmugapriya Kaminidevi, Thayumanavan Thangavelu, Jinu Udayabhanu, Senthil Murugan Thangavel. Antimicrobial activity of methanolic extracts of indigenous traditional Indian folk Medicinal Plant, *Gnaphalium polycaulon. Int J of Green Pharmacy*. 2015; IP: 223.30.225.254.
101. Seyyed Mansour Seyydnejad, Masumeh Niknejad, Ismaieel Darabpoor and Hossein Motamedi. Antibacterial activity of Hydroalcoholic Extract of *Calistemon citrinus* and *Albizia lebbeck*. *Am J of App Sci.* 2010; 7(1): 13-16.

102. Bauer, A.W., W.M. Kirby, J.C. Sherris and Turck, M. 1966. Antibiotic susceptibility testing by a standardized single disk method. Am.J.Clin.Pathol. 45 (4):493-496.
103. Yashodhakumar K, Philip J, Marcel J, Lutfun N, Satyajit D S. Screenings seeds of Scottish plants for antibacterial activity. *J of Ethno phar cology*. 2002; 83: 73-77.
104. Natchimuthu K, Mani J and Subbiah K. Synergistic Antibacterial of Four Medicinal Plants. *J of Plants Sci.7:32-38.* 2012; 1-4.
105. Bauer AW, WM Kirby, JC Sherris and Turck M. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol*. 1966 45 (4):493-496.
106. Punnagai K, Darling Chellathai D, Karthik V and Glory Josephine I. Evaluation of Antifungal Activity of Ethanolic Extract Of Andrographis Echioides An Invitro Study. *Int J of Pharma and Bio Sci.* 2016; 7(4): 6-10.
107. Odebiyi EO, Ramstard AH. Investigation photochemical screening and antimicrobial screening of extracts of *Tetracarpidium conophorum*. J Agric Chem Soc 1978;26:1‑7.
108. Bauer AW, WM Kirby, JC Sherris and Turck M. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol*. 1966 45 (4):493-496.
109. Sangh P, Saurabh K, Amit K, Neeraj K S, Jha K K. *Invitro* Anthelmintic Activity of *Luffa cylidrica* Leaves in Indian Adult Earthworm. *J of Pharmacognosy and Phytochemistry.* 2012; Vol 1: 27-30.
110. Javid Ahmad, Syed Tanveer and Bilar A Z. *In vitro* Anthelmintic activity of *Mentha longifolia*(L.) Leaves Against *Ascaridia galli. Global Veterinaria*. 2013; 11(1): 112-117.
111. Ponkshe and Indap. Evaluattion of *Invitro* Immunomodulatory activities of test plant extracts. 2002; 82-95.
112. Kalaichelvi K, Dhivya SM. Phytochemical screening and antibacterial activity of leaf extract of *Martynia annua* L. and *Premna latofolia,Roxb. J of Med Plants Studies*. 2016; 4(4): 84-87.
113. Shanmugapriya Kaminidevi, Thayumanavan Thangavelu, Jinu Udayabhanu, Senthil Murugan Thangavel. Antimicrobial activity of methanolic extracts of indigenous traditional Indian folk Medicinal Plant, *Gnaphalium polycaulon. Int J of Green Pharmacy*. 2015; IP: 223.30.225.254.

114. Yashodhakumar K, Philip J, Marcel J, Lutfun N, Satyajit D S. Screenings seeds of Scottish plants for antibacterial activity. *J of Ethno phar cology*. 2002; 83: 73-77.
115. Bauer AW, WM Kirby, JC Sherris and Turck M. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol*. 1966 45 (4):493-496.
116. Harison Masih, Jyotsna Kiran Peter and Pratima Tripathi. A Comparative evaluation of antifungal activity of medicinal plant extracts and chemical fungicides against four plant pathogens. *Int J Curr Microbiol App Sci*.2014;3(5): 97-109.
117. Nisha PV, Shruti N, Sweta Swamy K, Meera Kumari, Vedamurthy A B, Krishna V. Anthelmintic activity of *Pyrostegia venusta* using *Pheretima posthuma*. *Int J of Phar Sci and Drug Res*. 2012; 4(3): 205-208.
118. Antara Ghosh, Sujan Banik and Ariful Islan Md. *Invitri* thrombolytic, anthelmintic, antioxidant and cytotoxic activity with phytochemical screening of methanolic extract of *Xanthium indicum* Leaves. *A J of the Bangaladesh Phamacological Society*. 2015; 10: 854-859.
119. Vikhe *et al*., Edible Medicinal and Non medicinal Plants. 2013 Vol 10.
120. Tsai KD, Lin B R, Perng D S, Wei J C, Yu Y W, and Jaw Ming Cherng. Immunomodulatory effects of aqueous extract of *Ocimum basilicum(*Linn.) and some of its constituents on human immune cells. *J of Med plants Research*. 2011; 1873-1883: Vol 5(10).
121. Jitender K Malik, Manvi F V, Nanjwade, Alagawadi and Sanjiv Singh. Immunomodulatory activity of *gymnema sylvestre R.Br.* Leaves on *In vitro* human neutrophils. 2009; 2(8): 1284-1286.

ANNEXURE

VIVEKANDHA
EDUCATIONAL INSTITUTIONS

VIVEKANANDHA COLLEGE OF ARTS AND SCIENCES FOR WOMEN

[Autonomous]
SPONSORED BY : ANGAMMAL EDUCATIONAL TRUST.
An ISO 9001 : 2008 Certified Institution
(Approved by AICTE, Affiliated to Periyar University and
Re-accredited with 'A' grade by NAAC, Recognized under section 2(f) and 12(B) of UGC Act, 1956)
Elayampalayam - 637 205. Tiruchengode, Namakkal Dt., Tamil Nadu.
Phone : 04288 - 234560 (7 Lines), FAX : 04288 - 234033.

Date: 17.03.2017

The plant brought by **Ms.N.M.GAYATHRI,** II[nd] year M.Pharm, Department of Pharmacology, Swamy Vivekanandha College of Pharmacy, Elayampalayam, is identified as *Martynia annua*.L. belongs to the family Martyniaceae and *Pentanema indicum* (L.) Y.Ling. belongs to the family Asteraceae.

17/3/17

Dr.R.Prabakaran
Head of the Department of Botany
Vivekanandha College of Arts and Science for Women
(Autonomous)
Elayampalayam, Tiruchengode (Tk),
Namakkal Dt